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DONOR HEART PRESERVATION FOR HEART TRANSPLANTATION

DERECK RONALD WHEELDON

A Thesis submitted in partial fulfilment of the requirements of the Open University for the Degree of Doctor of Philosophy

February 1997

Sponsoring Establishment:
SmithKline Beecham Pharmaceuticals

Collaborating Establishment:
Papworth Hospital

Author no.: P 9159419
Date of anon: 4th September 1997

DECLARATION

I hereby declare that the work embodied in this thesis entitled "Donor Heart Preservation for Transplantation" is the result of my own investigations except where reference has been made to published literature or to specific input from named collaborators. The material used in this thesis has not been used in any other submission for an academic award.

This work has not been submitted in whole, or in part, for any other university degree or diploma.

Collaboration was obtained for the statistical analysis from Dr Linda Sharples (MRC Cambridge) with respect to the clinical studies, and from Dr Brian Bond for the laboratory-based studies. Dr Sally Darracott (Papworth) performed the Quantitative Birefringence measurements and Dr David Reid (Smith, Kline and Beecham) the NMR measurements.

While registered as a candidate for the degree of Doctor of Philosophy I have not been a registered candidate for another award of the Open University.

Dereck Wheeldon
Cambridge
February 1997

CONTENTS

Published Bibliography

ACKNOWLEDGEMENTS

I wish to offer my sincere thanks to the following:

Dr Alan Rothaul for his supervision of this project and for his valued advice, forbearance, support and encouragement.

Dr David Pegg for his external supervision of this work, advice and constructive criticism.

Sir Terence English for initiating this work and for encouraging an academic endeavour to parallel the pragmatic one.

John Wallwork, Director of the Transplant Unit, for allowing me to undertake this work and for lending his support.

The British Heart Foundation for funding part of this work with Grant Numbers : 870200, 88049 and 90038.

Dr. Sally Cankovic-Darracott for undertaking the histopathology.

Smith, Kline and Beecham for making the laboratory facilities available.

My medical and technical colleagues at Papworth, especially Dr Charles Potter, for their patience and willingness to cooperate, often at the most unsocial hours.

Jackie Yates, Sandie Hastings and Hazel Johnson for skilfully and patiently carrying out the huge task of producing the manuscript.

Dawn, my wife, for encouragement, patience and willingness to become a "thesis widow".

WHEELDON

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ABSTRACT

Heart transplantation has enjoyed a spectacular success over the past 25 years. Prior to 1980 less than 350 operations were carried out with an overall one year survival of less than 60%. In 1995 more than 3,000 transplants were performed with a one year survival of 83%. However, growth and improved survival have both plateaued over the last few years; the former because of the falling donor supply and the latter, in part, because of the use of less suitable donors in an effort to offset the problem of supply.

Much attention has been focused on the drama of the surgery and the intricacies of immunological manipulation whilst little effort has been devoted to the area of donor management, despite the fact that primary graft failure is responsible for as many post transplant deaths as either infection or rejection. Optimum preservation of the donor heart has also provided a difficult challenge, such that, despite a considerable scientific effort little advance has been achieved to extend the 4 hour safe storage limit which has remained in place over the past 20 years.

In this dissertation the problem has been approached by combining laboratory based preservation models with an objective regime of donor management. A sensitive isolated small animal working heart model was developed and used to characterise cardioplegic induction. Subsequently, the model was used to examine the interaction of oxygen content with the mode of delivery, during preservation.

Finally, a number of representative solutions were combined with the most promising oxygen delivery method. These studies served to illustrate the utility of controlled laboratory studies and offer the prospect of more than doubling post storage function.

The development of a rigorous donor management regime was also shown to be capable of reducing the variance in haemodynamic parameters by up to 44% whilst safely increasing the donor pool by approximately 30%. It is the contention of this thesis that the only prospect of improving the current impasse with the supply of donor hearts in sufficient quantity and of acceptable quality, is by the combination of appropriate laboratory models with controlled clinical trials.

CHAPTER 1

INTRODUCTION

1.1 HISTORICAL BACKGROUND

The concept of cardiac transplantation can be traced back to ancient myths and legends. However, the concept could only be turned into reality with the development of vascular surgical techniques in the early part of this century, cardiopulmonary bypass in 1954 and the means of managing rejection in the late 1960's.

Figure 1A Depiction of the first Heart Transplant from Chinese Mythology circa 500 BC

The first reported attempts at experimental heart transplantation were by Carrel and Guthrie in 1905[1], when they transplanted the heart of a puppy into the neck of a larger dog. Ventricular contractions started after about 1 hour of reperfusion and the heart continued to beat for a further 2 hours before intraventricular thrombosis forced the

experiment to be terminated. The crucial factor of donor coronary perfusion was simplified in 1933 when Mann[2] and co-workers developed a technique of cervical transplantation. Numerous investigators have subsequently used modifications of the Mann technique to study problems of heart transplantation, and the response of the denervated heart to pharmacological agents and physiological stress. Histological examination of the excised hearts demonstrated infiltration with lymphocytes, mononucleocytes and polymorphs, suggesting that graft failure resulted from undefined factors relating to tissue specificity rather than surgical technique. These were the first references to immunology and rejection[3].

Between 1940 and 1956 Demikhov[4], working in the Soviet Union, developed a canine intrathoracic heart transplant model which exhibited survival times of up to 32 days. The development of orthotopic models came in 1953 when Neptune[5] and others reported the transplantation of heart-lung blocks, using surface cooling and circulatory arrest of the recipient. In 1959 Cass and Brock[6] reported six attempts at autotransplantation and homotransplantation, where both atria were left intact in the recipient, thus simplifying the procedure. This procedure was described independently 1 year later at Stanford by Lower and Shumway[7], who obtained the first consistently successful results. With further modifications, made by Barnard[8], the technique is now used in most clinical centres as the basis for orthotopic heart transplantation.

These latter experiments were all conducted using the support of a pump-oxygenator system. In 1965 Kondo[9] and colleagues described a technique utilising profound hypothermia of both donor and recipient, by surface cooling. Transplantation took place during the 45 minutes of circulatory arrest. Following completion of the anastamoses, the recipient was rewarmed again using surface warming and warm

saline irrigation of the heart. This technique showed moderate success - one animal survived for 112 days - but was abandoned in favour of the more controlled conditions provided by the heart-lung machine.

By the mid - 1960's a considerable fund of knowledge had been acquired, particularly with respect to immunosuppression[10,11], and the increasing experimental success enjoyed by a number of groups world-wide, set the scene for the first human operation. One such group, at the University of Mississippi, attempted the first cardiac xenograft, when they transplanted a chimpanzee heart into the chest of a dying patient, in 1964[12]. Early function was good but the heart developed congestive failure shortly after weaning from the heart-lung machine.

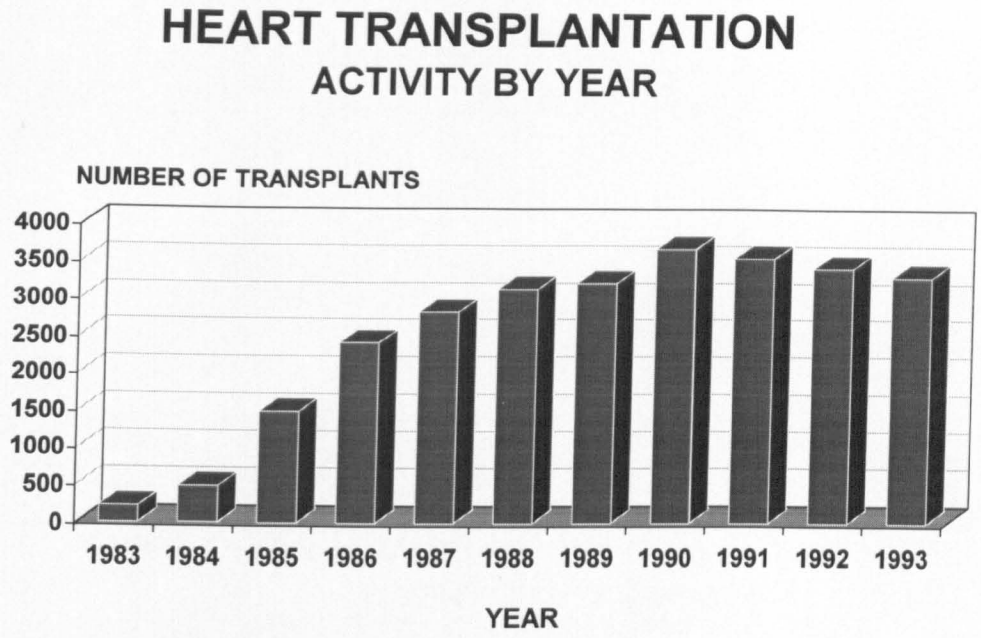
The first successful human heart transplant took place in Cape Town, unexpectedly, on the 3rd of December, 1967[13]. The patient was a 57 year old man with ischaemic heart disease. The surgery was successful and the heart functioned satisfactorily until the patient succumbed to a *Pseudomonas* pneumonitis on the 18th post operative day. At autopsy the heart showed signs of mild to moderate rejection. The first patient who could realistically be described as a *long term* survivor was operated on in Cape Town, 1 month later and lived an active life until he died from the hitherto undescribed complication of chronic rejection, eighteen months later[14].

The preservation technique used for this first and subsequent early procedures consisted of placing the donor on cardiopulmonary bypass and instituting whole body cooling, in the adjacent operating theatre to the one in which the recipient operation was taking place. The donor heart was removed when the core temperature reached 18°C and connected within minutes to a side limb of the bypass circuit used to support

the recipient. It was a further 10 years before distant donor heart procurement became feasible with the adoption of cold hyperkalaemic cardioplegia, developed for myocardial protection in routine cardiac surgery, together with hypothermic storage and transport of donor hearts[15]. This allowed a *safe* preservation time of some 4 hours, adding a considerable logistical challenge to the conduct of clinical heart transplantation.

This first pioneering operation initiated hectic activity in cardiac transplantation world-wide. Over the proceeding 12 months, 102 transplants were performed in 17 countries by 64 surgical teams. The results were appalling, with a mean survival of only 29 days. By the end of 1969, very few groups were persisting with active cardiac transplant programmes and in some countries this attitude was reinforced by a moratorium imposed by the healthcare providers. Some of those centres which persisted, most notably Stanford University, initiated a sustained research programme which achieved significant understanding and advances. The most significant of these was the development of an endomyocardial biopsy technique for the detection of rejection[16], the classification of rejection and the use of improved immunosuppressants, in particular Cyclosporin A[17]. At the same time a public education programme was launched to establish an awareness of the need for donor organs and the concept of brain death was ultimately established[18].

In the subsequent 25 years heart transplantation enjoyed a spectacular success, with overall activity increasing to more than 3,000 operations per year, with a 1 year survival rate of 83%[19].



International Registry 1994

Figure 1B Histogram showing the rapid increase in activity in the 1980s followed by decline in recent years as the donor supply became limiting.

However, both the activity and survival rates have remained static over the past few years, with the donor supply remaining the major limiting factor in the provision of transplantation as a more widespread therapy.

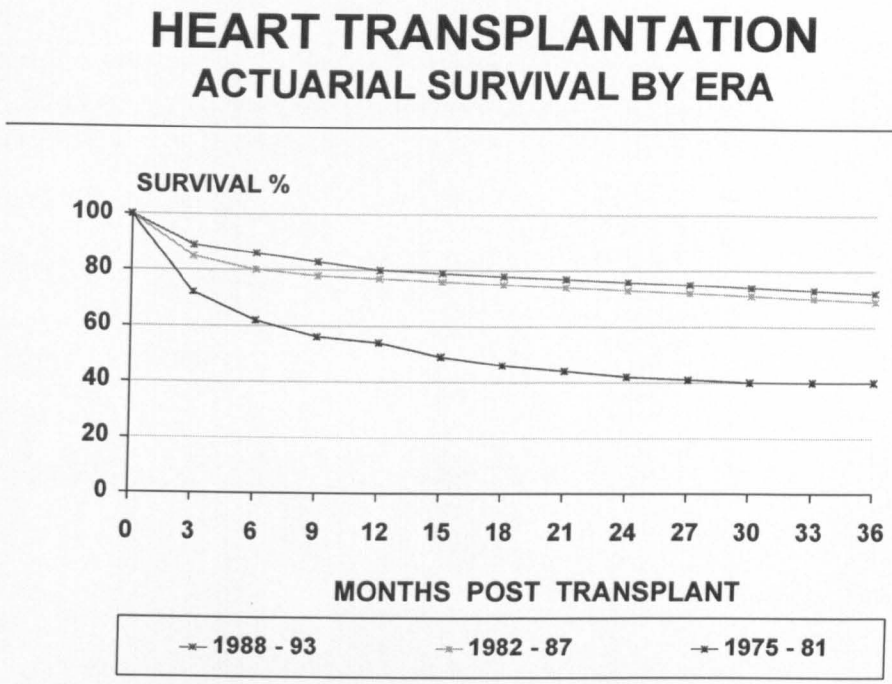


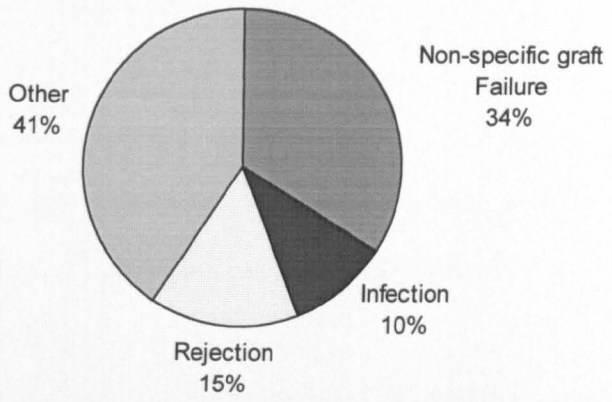
Figure 1C Actuarial survival in the six year eras between 1975 and 1993. Following an initial marked improvement in both acute and longer term survival, there has been little further improvement in recent years.

1.2 CURRENT STATUS

During the past 25 years much research effort has been focused on the surgical and immunological aspects of transplantation, with comparatively little attention being directed to the issue of optimal donor heart retrieval. This is somewhat surprising since data from the International Heart Transplant Registry has consistently shown that primary graft failure ranks on a par with both infection and rejection as an early cause of death[20].

CAUSES OF EARLY DEATH IN HEART TRANSPLANT RECIPIENTS

0 - 30 Days Post Transplant



ISHLT 1995

Figure 1D Pie Chart showing % Cause of Death 0-30 days post Transplant

The importance of good donor heart function, immediately following transplantation, cannot be over emphasised. During the course of cardiac transplantation, donor hearts undergo a sequence of five key events; initial donor management, cardioplegic induction, storage, global ischaemia during implantation, and reperfusion. Despite a plethora of published laboratory studies into improved donor heart preservation, little advance in the 4 hour safe preservation time, which has been the case for the past 20 years, has been achieved. The ISHLT Register[19] reports an Odds Ratio of 1.1 per hour of ischaemic time, up to 4 hours, after which there is insufficient data since most centres limit themselves to this time. A number of groups have shown, experimentally, improved metabolic preservation for up to 40 hours[21-30] by means of different solutions[22-24,26,27,29], by the use of perfusion systems[21,25] or by some form of

conditioning[30]. However, tentative clinical extensions of clinical preservation times have not met with success. This is in stark contrast to progress in the clinical preservation times achieved for other transplantable organs where kidneys are routinely stored for more than 36 hours[31] and livers for more than 12 hours[32].

There are probably two major reasons for this discrepancy; firstly donor hearts are required to perform a high level of work within a short time of implantation and secondly there are some significant differences in the metabolic features, with respect to both energy pathways[33] and natural defence mechanisms[34,35] of the myocardium.

The heart contains a number of disparate tissue subtypes - contractile, conductive, connective and vascular, all of which have different susceptibilities to tissue injury. Whilst the majority of investigators have focused on the quantitatively dominant contractile tissue, failure of electrical conduction or vascular performance is equally crucial in this dynamic organ. Equally important, the biochemical and biophysical reactions within these tissues show heterogeneous responses to cooling. Whilst cellular energy processes such as phosphorylation, may decline rapidly, and active cation transport in particular, processes involving diffusion may be reduced to a lesser extent, and this is further compounded by the phase transitions which cell membranes undergo, in response to a decrease in temperature.

The heart can be viewed as a chemodynamic machine which liberates energy stored in chemical bonds to perform mechanical work. The main substrates from which this chemical energy is liberated, are fatty acids, glucose, pyruvate and lactate, which are broken down into 2-carbon fragments which enter the Krebs cycle. Hence the heart is essentially an aerobic organ, rich in the respiratory enzymes, with limited energy

storage capacity, a high energy requirement and an intolerance for toxic metabolites, including protons. This makes the organ peculiarly susceptible to ischaemic damage.

There is now convincing evidence that oxygen-derived free radicals are important mediators of myocardial damage[34] resulting from the ischaemia/reperfusion sequence. Free radical production is consequently expected to occur in the course of the transplantation procedure in which hearts are subjected to two superimposed periods of global ischaemia. Thiol groups, linked either to the tripeptide glutathione or to various proteins, play a key role in the defence of tissues against oxygen toxicity. In the heart there is a shift of the cellular thiol pool towards oxidation, leaving the myocardium more vulnerable to free radical damage. Curiously there is also a marked reduction in Defence Antagonist Factor (DAF) in myocardial tissues[35]. Since DAF provides a natural defence against autologous complement attack on tissues, and many of the processes involved in organ retrieval and subsequent transplantation are known to activate complement, this leaves the heart in a particularly vulnerable state. All of the above does not mean that the problem is intractable, merely that it is difficult and requires an individual solution.

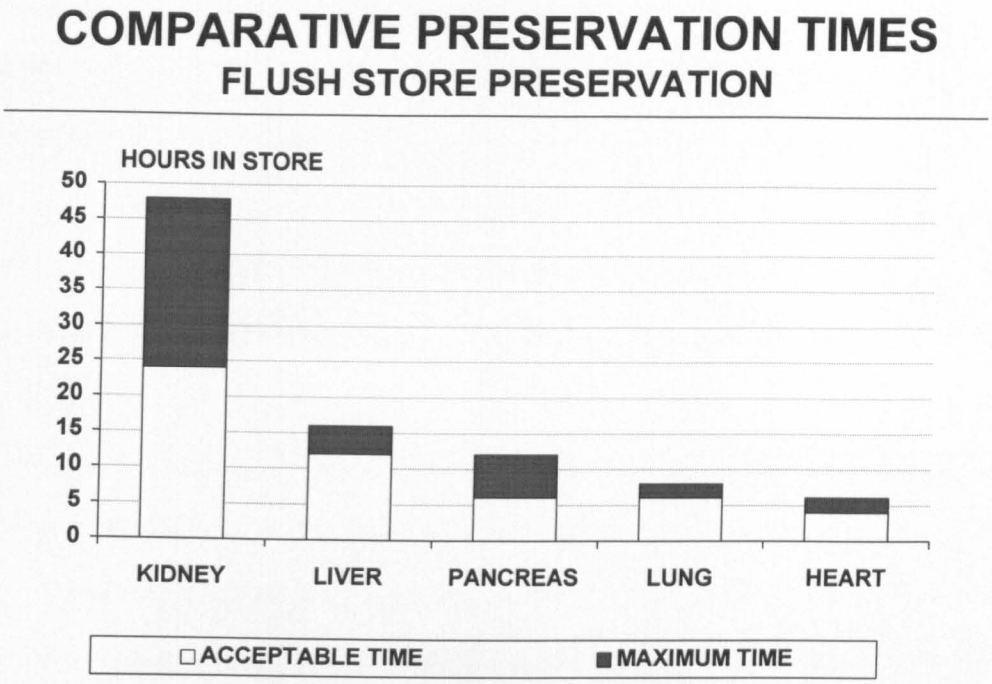


Figure 1E Comparative preservation times for solid organs[36]

It is somewhat surprising to note that there is currently no specifically designed cardiac transplant preservation solution or technique available. Each centre has merely adopted techniques used in their routine cardiac practice, for donor heart preservation. Since the conditions prevailing during donor heart preservation are very different to those in routine heart surgery, it is not altogether surprising that these techniques are not very efficient or reliable. In order to establish what the range of techniques are and to get some idea of what differences there may be in the clinical outcomes associated with these techniques, the author undertook an international survey (Appendix A)[37]. This examined the issues of; donor pre-treatment, cardioplegic induction, donor heart storage methods, transport medium and reperfusion techniques. Whilst the results of this survey were somewhat compromised by the availability of Centre outcomes only, rather than individual patient

outcomes, it did reveal the wide disparity in techniques currently employed and gave some pointers to those which may hold out the promise of improved functional preservation.

Twenty-seven percent of respondents reported using some form of donor pre-treatment. More than 90% reported using a single flush cardioplegic induction with the use of eight different generic types of cardioplegic solution, only 5% of which were oxygenated. Six different types of storage media were used and melting ice was the coolant used by 66% of Centres. Storage temperatures ranged between 0 - 7° C, with 78% of respondents reporting the use of 4° C cooling. Some 55% of Centres reported the use of some form of reperfusion modification. There was an overall 30 day mortality rate of 9.6%. The only clear outcome from this survey was that saline provided better functional preservation than other solutions, as a storage medium, and a suggestion of a trend towards benefits from multiple dose blood cardioplegia. Following the publication of this survey, the International Society now routinely collects some of this donor related data as part of the Registry.

The results of the survey strengthened the anecdotal impression that donor heart preservation is being carried out using the default methods borrowed from routine cardiac surgery, albeit with a few modifications in some cases. The systems employed pay scant regard for the different conditions prevailing during heart storage and rely mostly on the metabolic benefits of hypothermia, without taking into account the potential harmful effects of low temperature flush/store techniques[38]. A default 4° C temperature is also employed mainly because of the convenience of using melting ice rather than for any good scientific reason. Thus the data obtained from the Registry pointed to the serious consequences of inadequate donor heart retrieval and the

survey demonstrated the diversity of preservation methods in current clinical use and the lack of any transplant specific technique.

1.3 PROSPECTS FOR IMPROVED DONOR HEART PRESERVATION

It has already been pointed out above, that the donor supply currently represents the most significant factor limiting the availability of heart transplantation as a therapy. In addition it would seem that the supply is actually diminishing, due principally to changes in road safety measures, and is only likely to be minimally improved by changes in social attitudes to transplantation. It is therefore imperative that the best use is made of this scarce resource.

An issue which has received little attention, is that of donor management. There is a sparse but significant literature, going back to the 1960's which links brain injury to myocardial dysfunction and even necrosis[39-41] and this subject is comprehensively covered in Chapter 2. Anyone involved in the practical business of organ retrieval will also attest to the practical difficulties in managing brain-dead donors, due to their haemodynamic and metabolic instability. Following close correspondence with previous colleagues working with animal models of brain death in Cape Town[42], the author proposed a series of clinical studies aimed at improving peri-operative donor heart function, with the goal of improving the retrieval rate and the functional capacity of donor hearts *prior* to preservation.

Taking into account the results of the survey, it seems clear that little of the large body of laboratory based research into improved methods of preservation, has become established in clinical transplant practice. This suggests that the models used do not reflect the clinical situation and/or that the effects of clinical brain death and

subsequent management overshadow any advantage which may potentially be offered by improved preservation techniques. The author therefore decided to address the problem from two distinct but complimentary approaches; that of improved clinical management and the establishment of improved laboratory models.

1.4 AIMS

The aims of this thesis are:

a) To develop a method for characterising donor heart function in the donor and using this to guide donor management, with the object of producing hearts with optimal function, prior to excision and storage.

b) To develop and refine laboratory models for developing and evaluating potentially improved cardiac preservation techniques.

c) Identify potential solutions and techniques which might offer the prospect of improved preservation by critically appraising current clinical methodologies.

d) To explore the potential for combining these clinical and laboratory methods with the object of realising an advance in the efficacy and safety of current clinical donor heart preservation methods.

1.5 ORGANISATION OF THESIS

Since the underlying premise behind this thesis is that a synthesis of laboratory and clinical studies holds out the best prospect of making a significant advance, it follows that the organisation of the work should be similarly organised.

In ***Part 1*** the issues of brain death and donor management are discussed along with the results of the implementation of specific interventions in the management of clinical cardiac donors. The development of a rigorous donor management regime was shown to be capable of reducing the variance in haemodynamic function in donors, prior to excision, by up to 44%, whilst allowing the safe retrieval rate to be increased by up to 30%[43] (Chapter 3).

In ***Part 2*** a number of laboratory based models and studies are described, leading to a proposal for achieving a real advance in donor heart preservation by the *combination* of these two elements. A sensitive small animal working heart model was developed which was then adapted into a large animal and/or human working heart model to provide a pre-clinical screening device (Chapter 4). The small animal working heart model was also used to develop and characterise cardioplegic induction (Chapter 5). The model was further used to show that the combination of a method for enhanced oxygen delivery together with an improved preservation solution, holds out the prospect of more than doubling post storage function, compared with the current clinical technique (Chapter 6). Subsequently the model was also used to demonstrate it's potential as a screening method for a number of different techniques and solutions to provide improved donor heart functional preservation, during storage (Chapter 7).

In the General Discussion, the author has attempted to bring together the knowledge gained from these two arenas of study and suggest some synergies and potentially fruitful avenues of further investigation.

1.6 COLLABORATION

Since much of the work described in Part 1 of this thesis is of a clinical nature, the author was assisted in his endeavours by the clinical team. This team of surgeons, anaesthetists and technicians tended to change every six months or so, as members rotated through the service. The author was responsible for proposing, initiating and sustaining these studies, with the financial assistance of a number of British Heart Foundation Grants (of which the author was the grant holder), as detailed in the Acknowledgements. Statistical advice was also obtained, as acknowledged. The Survey was conducted under the auspices of the International Society and the two senior surgeons at Papworth, however the work was undertaken by the author.

1.7 SUMMARY

The stimulus for this thesis arose out of the frustrations experienced by the author, with the inadequacies of current methods for donor heart preservation, as experienced during more than 20 years of working in the field. The nature of the problems encountered would appear to be of two distinct kinds; those related to the management of brain-dead donors and the condition of donor hearts prior to preservation, and secondly those aspects which relate to preservation techniques, *per se*. The studies described in this thesis were therefore undertaken in both the clinical setting and in the laboratory.

Central to this thesis, therefore, is the contention that the best prospect of improving the current impasse with the supply of human donor hearts, in numbers which come closer to satisfying demand, and of a quality which allows rapid rehabilitation of the recipient, is by the *combination* of appropriate laboratory models with improved clinical donor management techniques. It is the opinion of the author that the work described

in this thesis demonstrates the feasibility of achieving a significant improvement over the current status of heart preservation for transplantation.

CHAPTER 2

BRAIN DEATH AND DONOR MANAGEMENT

2.1 BACKGROUND

Patients with irreversible brain injury constitute the main source of donor organ material. There is, however, extensive clinical and experimental evidence to show that the process of brain death results in cardiac damage. The adverse effects of brain death on the heart were demonstrated as early as 1954[44] when subendocardial haemorrhages in the myocardium of patients dying from intracranial lesions was noted. More recent studies in both experimental animals[45,46] and in clinical donors[47,48] have suggested that brain death has major histopathological and functional effects. Consequently, without specific intervention, circulatory collapse will usually take place within 72 hours of brain death[49].

Two major effects of brain death have been observed in experimental studies. The first is a series of major electrocardiographic, haemodynamic and histopathological changes which take place during and immediately following the agonal period[46,39,50-53]. The second consists of significant endocrine changes, which in turn result in major metabolic changes[46, 39,50-53].

A sudden increase in intracranial pressure or the sudden onset of cerebral ischaemia leads to a series of major pathophysiological changes which have collectively been referred to as the *autonomic storm*[39,51,54]. The immediate consequence of brain death is a predominance of parasympathetic activity manifested by bradycardia progressing

to sinus standstill. This is then followed by a sinus tachycardia and multiple ectopic activity of multifocal origins as the sympathetic system dominates[39,55,56]. This is usually followed by a further period of sinus tachycardia accompanied by marked ischaemic changes, probably induced by endogenous catecholamine release, coronary artery spasm and high systemic vascular resistance (SVR) induced by increased sympathetic activity. This ischaemia frequently results in some myocardial damage, especially in the subendocardium. The sequence then settles down to a bradycardia, often with dysrhythmias, associated with the loss of myocardial energy stores[54].

The haemodynamic changes during this process reflect the body's attempts to compensate for the intracranial changes taking place during *coning*[55] (Cushing's reflex). Significant and often massive increases in SVR, caused by the sympathetically induced peripheral vasoconstriction, brings about a redistribution of blood volume into the capacitance vessels, leading to a rapid accumulation within the great veins and right atrium. The right ventricle is usually able to adjust to this acute increase in preload whilst the left is not, often giving rise to complete standstill in pulmonary blood flow[53]. This is probably the genesis of the so called *neurogenic pulmonary oedema.*

In approximately 75% of experimental animals undergoing brain death, histopathological changes develop in the left ventricular wall[52]. These consist of contraction bands, focal coagulative necrosis and myocytolysis. This myocardial damage may be reflected in electrocardiographic abnormalities, manifested as *pseudo infarct* patterns, the most serious of which are *Q* waves and *J* waves, where there is concomitant hypothermia[56]. Histopathological examination usually reveals widespread injury even in hearts which do not exhibit such ECG changes[39,57-61]. Animal studies have pointed toward an association between the mode of death and high energy

substrate levels[57] and there is also evidence that the more acute and the greater the rise in intracranial pressure prior to brain death, the greater is the catecholamine release, and the more severe is the resulting impairment of myocardial function[59].

In animal studies the thyroid hormones, plasma free triiodothyronine (T3) and thyroxine (T4), together with plasma cortisol and insulin, fall significantly within a few hours of the onset of brain death[50]. Antidiuretic hormone (ADH) also disappears from the plasma within a few hours. Whilst ADH is not crucial to the maintenance of vascular tone in normal patients, the absence of vasomotor impulses in brain death would seem to result in a dependency on ADH. These changes in circulating hormones are associated with a reduction in myocardial energy stores as well as a significant increase in myocardial lactate[54]. In addition, functional testing of these hearts demonstrates a deterioration in myocardial function as evidenced by significant reductions in cardiac output, stroke volume, and left ventricular developed pressure[60]. These findings of abnormal hormonal changes and anaerobic metabolism have been confirmed in human donors[61] although the correlation with function has been less clear.

In 1982, following acute graft failure in two recipients who had received hearts from apparently normal donors, a study of myocardial preservation during transplantation was initiated at Papworth Hospital in an effort to determine whether this failure had resulted from inadequate myocardial protection during storage or from some other cause. The primary determinants of myocardial function used in this study were; quantitative birefringence measurements (an indirect measurement of myocardial contractility) on biopsy specimens[62] haemodynamic function, and survival. Of 172

human donor hearts studied just prior to excision, biopsy assessment revealed significant myocardial injury in 73 (42%)[63] of these hearts.

Following their studies of experimental brain death, Novitzky and Cooper demonstrated that HRT was associated with a return to aerobic metabolism and myocardial functional improvement[47,50,52,55,64-67]. However, HRT was subsequently investigated by a number of other investigators[66-71] with controversial findings. In all of these studies an understanding of the possible contribution of HRT to outcomes has been difficult to ascertain because of the lack of a convenient objective measure of cardiac function during the retrieval procedure.

The effects of appropriate haemodynamic donor management have become clearer in recent years[72-74]. In a study of brain-dead humans a Japanese group were able to demonstrate a 20 fold increase in physiological survival with the use of arginine vasopressin and noradrenaline[49], to maintain homeostasis.

The use of HRT and more specific donor management techniques seemed, to the author, to be a potentially useful approach. The following series of studies was therefore devised to explore and characterise a method for improving the number and quality of retrieved donor hearts.

2.2 THE PILOT STUDIES

Patients and Methods

All donors were brain dead, on ventilatory support and with no transplant specific contra-indications (age, viral disease, infection) in their recent medical histories. Any incidence of hypotension had been corrected with fluid replacement and/or inotropic

support. The age, sex, cause of death and duration of ventilatory support of the donors is shown in Table 2.1.

TABLE 2.1

Comparison of Donor Data in the Four Pilot Study Groups

GROUP	SEX M F	AGE Years	CAUSE OF DEATH T H O	ISCH TIME Mins	VENTILATOR Hrs
A n=12	9 3	30(17-39)	8 3 1	169	58 (16-130)
B n=12	10 2	28(17-42)	8 3 1	170	79 (19-160)
C n=6	2 4	29(16-43)	2 3 1	154	65 (24-170)
D n=12	8 4	26(13-47)	5 4 3	178	103 (2-240)

Group **A** = Control Group. Group **B** = Bolus T_3 (2 μg/hr)
Group **C** = Bolus T_3 (4 μg/15 mins) Group **D** = Bolus + Infusion
Causes of Death:
T = Trauma
O = Other (Respiratory arrest, overdose, viral meningitis, tumour or neurological operative death)
H = Sub-arachnoid Haemorrhage
ISCH TIME = Ischaemic Time

Study 1

Study of the Effect of Hormonal Pre-treatment Therapy (as described by Novitzky and Cooper (2μg T_3, Cortisol and Insulin)

In this prospective randomised study, 24 donors were separated into two groups:

Group A Twelve donors formed the untreated control group.

Group B Twelve donors received hourly intravenous bolus infusions of triiodothyronine (2 μg T_3) and cortisol (100 mg) with blood glucose

levels assessed half-hourly by the Dextrostix test and normalised with Insulin.

Donors received 2-3 infusions according to the duration of multi-organ retrieval; 4 donors received 2 infusions, 8 donors received 3 infusions.

Additionally, in accordance with standard protocol at the time, all donors received 1g Methylprednisolone (as a membrane stabiliser)[74] in addition to the hormonal pre-treatment described above.

Novitsky and Cooper reported on a sequential study in which 21 HRT donors were compared with 26 previous donors in whom all other management strategies remained unchanged. The HRT group received hourly bolus injections of 2 μg T3, 100 mg cortisol and 10 - 30 IU of insulin for between 3 - 8 hours prior to organ retrieval. There was a return to normal serum levels of T3, cortisol and insulin in the HRT group which also demonstrated a 53% increase in arterial pressure, a 35% decrease in central venous pressure and an 88% decrease in inotropic requirement. All 21 donor hearts were successfully transplanted[62].

Study 2

Study of the Effect of an Increased Dosage of T_3

In this non-randomised study the effects of increasing the T_3 dosage, in an attempt to normalise serum levels, was explored.

Group C Six consecutive donors received a bolus injection of 4 μg T_3 every 15 minutes (up to a maximum of 20 μg) and blood glucose levels were normalised with insulin half-hourly.

Study 3

Study of the effect of a bolus followed by an infusion of T_3

In this non-randomised study, the effects of T_3 combining a loading dose of T_3 followed by a continuous infusion, was studied.

Group D Twelve consecutive donors received a bolus injection of 4 μg T_3 followed by a continuous infusion at 4 μg/hr and insulin was given as described in Group C.

Assessment of the Effect of Hormonal Pre-treatment

On arrival at the donor hospital, blood samples were obtained hourly from all donors for the assessment of free T_3, plasma cortisol and plasma lactate. Inotropic support was given to maintain the mean arterial pressure above 60 mmHg with a central venous pressure of 6-10 mmHg. The requirement for inotropic support (Dopamine) of the donor heart both before and after transplantation was compared in all groups.

Haemodynamic status of recipients was compared in Groups A and B by the following assessments: **Cardiac index (CI)**

Stroke Work Index (SWI)

Pulmonary Vascular Resistance (PVR)

Cardiac outputs were measured by thermodilution (mean of three measurements) and cardiac pressures by conventional fluid filled manometer lines connected to *Medex* transducers and *Hellige* pressure amplifiers.

CI was defined as:

Cardiac Output/Body Surface Area ($l.min^{-1}.m^{-2}$).

SWI was defined as:

Stroke Volume Index x (Mean Aortic - Mean Central Venous Pressure) x 0.0136 $g.m.m^{-2}$.

PVR was defined as:

(Mean Pulmonary Artery Pressure - Mean Pulmonary Capillary Wedge Pressure)/Cardiac Output x 79.9 ($dynes.sec.cm^{-5}$).

Biopsy assessments were made at intervals during, and one week and one month following, transplantation to compare the effect of pre-treatment on myocardial function in the treated and the control groups.

Biopsy Assessment

The technique of Quantitative Birefringence Measurements (QBM) is one which had been developed by one of my colleagues (Dr. Darracott) as a tool for monitoring intra-operative myocardial protection[75]. Assessment by QBM is based on the contractile response of muscle fibres in myocardial biopsies, to added ATP and calcium, and the effect this has on transmitted polarised light. This test was selected because clinically and experimentally it had been shown to correlate with physiological assessment at cardiac catheterization, to be capable of monitoring myocardial protection during open heart surgery[76] and transplantation[77] and to provide a reliable index of postoperative myocardial function[78]. We had also been

monitoring donor hearts for some years with QBM, with the intention of investigating the potential for making the technique portable.

Biopsy Sequence

Biopsies were taken from the apex of the left ventricle with a Tru-cut biopsy needle at 5 time intervals during transplantation:

1) prior to donor heart excision
2) after cardioplegic arrest with St Thomas` cardioplegia solution and subsequent excision
3) on arrival at the recipient hospital following storage and transport
4) at the end of transplantation before removal of the aortic clamp
5) before the recipient chest was closed (Reperfusion).

Right ventricular endomyocardial biopsies were taken 1 week and 1 month following transplantation (biopsies 6 and 7), at the same time as biopsies were taken for rejection monitoring.

All recipients received similar anti-rejection therapy comprising Cyclosporin A, Azathioprine and steroids.

Preparation of Specimens

The biopsies were immediately frozen in liquid nitrogen and stored for up to 4 weeks at -70^0C. The specimens were then sectioned in a cryostat at -30^0C. The birefringence of the muscle fibres in freshly cut sections was measured under a polarising microscope before and after the addition of ATP and calcium[62].

Grading of Biopsy Specimens

In normal muscle there is a great increase in birefringence following the addition of ATP and calcium due to increased orientation of the myosin micelles as the muscle fibres contract. In damaged or ischaemic muscle, this response is diminished. As in previous studies, donor hearts with a birefringence ratio of 1.15 or less were classed as *Poor* and those above 1.40 as *Good*[76,77].

Statistical Analysis

Results are expressed as means and standard errors of the mean (SEM) or frequencies for categorical variables. Analyses of the changes in QBM, T_3, cortisol, lactate and glucose levels, through time within the groups were done with paired Students` t-tests. For Study data concerning $T_3$, cortisol, lactate, glucose levels and haemodynamic measurements were compared between the groups by unpaired Students` t-test. Data concerning pre- and postoperative inotropic support of the donor hearts was analysed by the McNemar test for paired data. Comparison of QBM results and T_3 levels among the four groups at each time point was done by a One-Way Analysis of Variance.

2.3 RESULTS

Study 1 ***(The infusion of 2 μg T_3, 100 mg cortisol and insulin)***

Groups A and B: 12 untreated and 12 pre-treated donors.

Free T_3

Initially, 19 of the 24 donors in this study had T_3 levels below the normal range (3.1 to 8.0 pg/ml). At excision the T_3 level had improved in only one pre-treated donor in

Group B (Table 2.2). Mean plasma T_3 levels remained subnormal in both Group A and Group B (1.3 ± 0.5 and 2.6 ± 0.6 pg/ml) respectively, (Table 2.2).

TABLE 2.2

The Effects of the Various Thyroxine Regimes on Serum Levels of T_3

GROUPS	T_3 pg/ml MEAN (SEM)	CORTISOL nmol/l MEAN (SEM)	LACTATE mmol/l MEAN (SEM)	GLUCOSE mmol/l MEAN (SEM)
Group A				
Initial	1.5 (0.3)	661 (225)	2.0 (0.3)	8.5 (1.5)
Final	1.3 (0.5)	1277 (151)	2.6 (0.5)	10.9 (2.2)
Group B				
Initial	2.1 (0.3)	271 (61)	2.3 (0.5)	8.8 (1.0)
Final	2.6 (0.6)	1057 (150)	2.6 (0.4)	7.8 (1.5)
Normal Values	3.1 - 8.0	220 - 770	0.6 - 2.4	2.5 - 6.6

Plasma Cortisol

At the beginning of this study there was a wide variation in plasma cortisol in the donors (28-2000 nmol/l, normal range 220-770 nmol/l) probably due to the fact that some had been treated with steroids in the ICU. Unexpectedly, during the study period control Group A showed an increase in plasma cortisol levels similar to that of pre-treated Group B. Since all of the donors had routinely received Methylprednisolone it is likely that this unexpected increase was due to the effects of this glucocorticoid. It was therefore difficult to separately assess the effect of pre-treatment with cortisol.

Plasma Lactate

Levels rose slightly in both groups, a mean of 2.0 ± 0.3 to 2.6 ± 0.5 mmol/l in Group A and 2.3 ± 0.5 to 2.6 ± 0.4 mmol/l in Group B. Since both groups had a degree of lactic acidosis, at the time of excision, this suggests a degree of anaerobic metabolism.

Blood Glucose

Control values were slightly elevated in both groups 8.4 ± 1.5 and 8.8 ± 1.0 mmol/l respectively. Levels rose to 10.9 ± 2.2 mmol/l in control Group A at excision and were slightly above normal in pre-treatment Group B; 7.8 ± 1.5 mmol/l. The difference in mean change between the two groups was not significant ($p = 0.106$).

Requirement for Inotropic Support

Before excision: 8 donors in each group were on dopamine support (Table 2.3). The mean dosage in control Group A increased from 5.9 ± 1.4 to 9.5 ± 3.1 $\mu g.kg^{-1}.min^{-1}$ by completion of excision. In contrast, only 3 donors in pre-treatment Group B continued to need support at a mean dosage of 3.3 ± 0.7 1 $\mu g.kg^{-1}.min^{-1}$. These differences were significant with respect to both the numbers and the dosage ($p<0.05$).

Following transplantation: The improvement seen in Group B was not maintained post transplant since 6/12 needed dopamine support (3.0 ± 0.5 $\mu g.kg^{-1}.min^{-1}$). However, the requirement in Group A had improved since only 2/12 required support (3 and 8 $\mu g.kg^{-1}.min^{-1}$ respectively). This difference was not significant.

TABLE 2.3

Comparison between the Pilot Study Groups with Respect to Inotrope Requirement

	GROUP A	GROUP B	GROUP C	GROUP D
Initial Numbers	8/12	8/12	5/6	8/12
Mean Dose (SEM)	5.9 (1.4)	3.9 (0.4)	5.0 (0)	6.0 (1.2)
Range	1 - 14	2 - 5		2 - 12
Explant Numbers	8/12	3/12	4/6	4/12
Mean Dose (SEM)	9.5 (3.1)	3.3 (0.7)*	4.5 (1.0)	3.3 (0.8)
Range	1 - 20	2 - 4	3 - 7	1 - 5
Recipient Numbers	2/12	6/12	3/6	1/12
Mean Dose (SEM)	4.0 (1.0)	3.0 (0.5)	4.0 (1.0)	6.0 (0)
Range	3 - 5	2 - 4	2 - 5	

* $p<0.05$

This table shows the differences in both the numbers of patients requiring Dopamine support at the beginning and the end of the retrieval, and the differences in Dopamine dose requirements ($\mu g.kg^{-1}.min^{-1}$). The last row shows the impact of this on their respective recipients

Haemodynamic Measurements

Following transplantation there were no significant differences in Cardiac Index (CI), Stroke Work Index (SWI) or Pulmonary Vascular Resistance (PVR) between the treated and untreated hearts. These parameters were chosen as they represent classical clinical indices of cardiac function and load. The PVR is particularly significant in cardiac transplantation because of the impact of increased loads on the post-ischaemic right ventricle.

TABLE 2.4

Recipient Haemodynamic Function

GROUPS	CI $l.m^{-1}.m^{-2}$	SWI $g.m.m^{-2}$	PVR dynes.sec.cm^{-5}
Group A	3.38	22.49	206.4
Range	2.0 - 4.6	17.7 - 38.0	136 - 272
Group B	3.23	28.52	175.2
Range	1.9 - 4.7	12.7 - 32.4	72 - 280
Normal Values	3.6	30	64

This table shows the post transplant function of the two groups of donor hearts. There were no statistical differences between the groups.

Biopsy Assessment

Figure 2A illustrates the typical trend observed in the earlier studies of deterioration throughout the peri-operative procedure, with some recovery during cardiac rewarming and at chest closure. However, it is clear that there were significant differences between the treatment groups:

At excision: Myocardial function as assessed by QBM was similar in Groups A and B 1.31 ± 0.04 and 1.31 ± 0.06 respectively.

After transport: Both groups deteriorated significantly during storage to 1.15 ± 0.04 ($p < 0.005$) and 1.19 ± 0.05 ($p < 0.01$) respectively. However, whilst there was some recovery in Group A by the final biopsy (mean value 1.25 ± 0.04), the deterioration in Group B continued to the end of implantation (deteriorated from 1.28 ± 0.05 at the onset of storage to 1.16 ± 0.03 at the end of implantation, $p < 0.002$). The depressed myocardial function detected in these hearts by QBM at the end of

implantation was reflected by the fact that 6 needed inotropic support before weaning off cardiopulmonary bypass, compared with only 2 in Group A. However, Group B hearts had also shown significant improvement by the final biopsy (mean value 1.22 ± 0.04, p = 0.05). Ischaemic times were similar in Groups A (169, range 95 - 223 mins) and B (171, range 127 - 217 mins), p = 0.413.

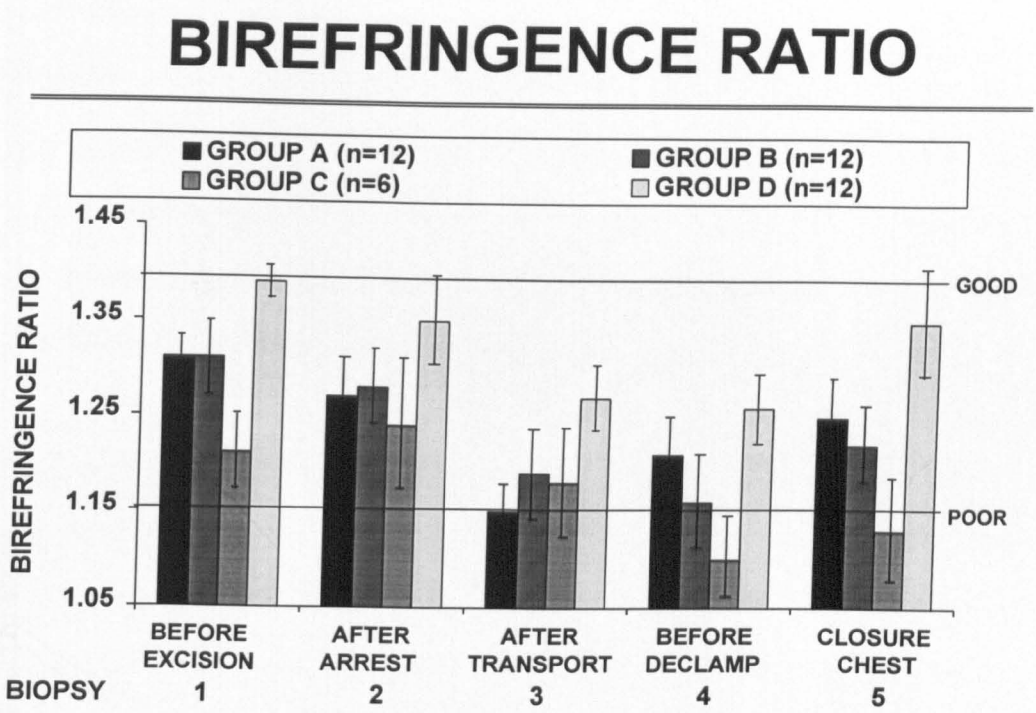


Figure 2A A histogram depicting the changes in QBM during the five peri-operative stages of heart transplantation, in the four groups; **A** - Controls, **B** - Bolus 2 μg, **C** - Bolus 4 μg, **D** - Infusion. All hearts show a decrease in QBM with ischaemia. This is most evident in the high T3 dose group (**C**). A clear improvement is seen with the constant infusion group (**D**), which is sustained throughout the transplant procedure.

Study 2 ***(The effect of an increased dosage of T_3)***

Since the previous study had shown that a bolus of 2 μg/hr of T_3 was insufficient to normalise plasma T_3 levels in most donors, in contrast to the results reported by Novitzky et al[50,60,61,79], the effect of increasing the dosage of T_3 was tested.

(Group C) Intermittent boluses of 4 μg T_3 administered every 15 minutes

Initial free T_3 levels were subnormal in all 6 donors in this group (mean value 1.65 ± 0.32 pg/ml). After the first bolus 3 donors had T_3 levels within the normal range (mean 5.17 ± 1.2 pg/ml) and remained normal. A fourth donor had normal levels after the third bolus. The remaining two failed to normalise even after cumulative doses of 16 μg and 20 μg, the maximum doses given. QBM failed to reveal any beneficial effect of hormonal pre-treatment (mean value at excision 1.21 ± 0.07, Figure 2A). Only 4 hearts were biopsied after implantation but, as in Group B, these hearts deteriorated throughout the period of ischaemia (157 ± 19, 120 - 200 mins) to a mean value of 1.10 ± 0.06. Three of these hearts needed inotropic support after implantation (Table 2.4). Biopsies at the end of transplantation were incomplete (inadequate specimens (2), not attempted (2)).

Study 3 ***(Group D) A bolus injection of 4 μg T_3 followed by an infusion of 4μg /hr.***

Samples for the assessment of free T_3 were obtained from only 9 of the 12 consecutive donors studied. One had normal free T_3 levels before treatment and a further 2 normalised following treatment. At the beginning of the study eight donors were receiving dopamine support, mean dose 6 ± 1.2 $\mu g.kg^{-1}.min^{-1}$ which was reduced to 4 who were receiving 3 ± 0.8 $\mu g.kg^{-1}.min^{-1}$ at excision. QBM indicated good myocardial function in these hearts at excision (mean value 1.39 ± 0.05) and deterioration during ischaemia (166 ± 12, 91 - 219 mins) only just reached significance (mean value 1.23 ± 0.04, $p = 0.05$). Only one heart needed inotropic support at the end of implantation and QBM in the final biopsies (mean 1.35 ± 0.06) were not significantly different from QBM at excision, ($p = 0.92$).

Biopsy Assessment of Myocardial Function Following Transplantation

QBM of endomyocardial biopsies taken 1 week and 1 month after transplantation were compared with values in biopsies taken at transplantation to see whether there were any differences in the groups. These results are illustrated in Figure 2B, which includes mean values for the previous 172 donors, for comparison.

In all groups QBM one week and one month after transplantation were not significantly different from the control biopsy (1) taken from the donor heart before excision.

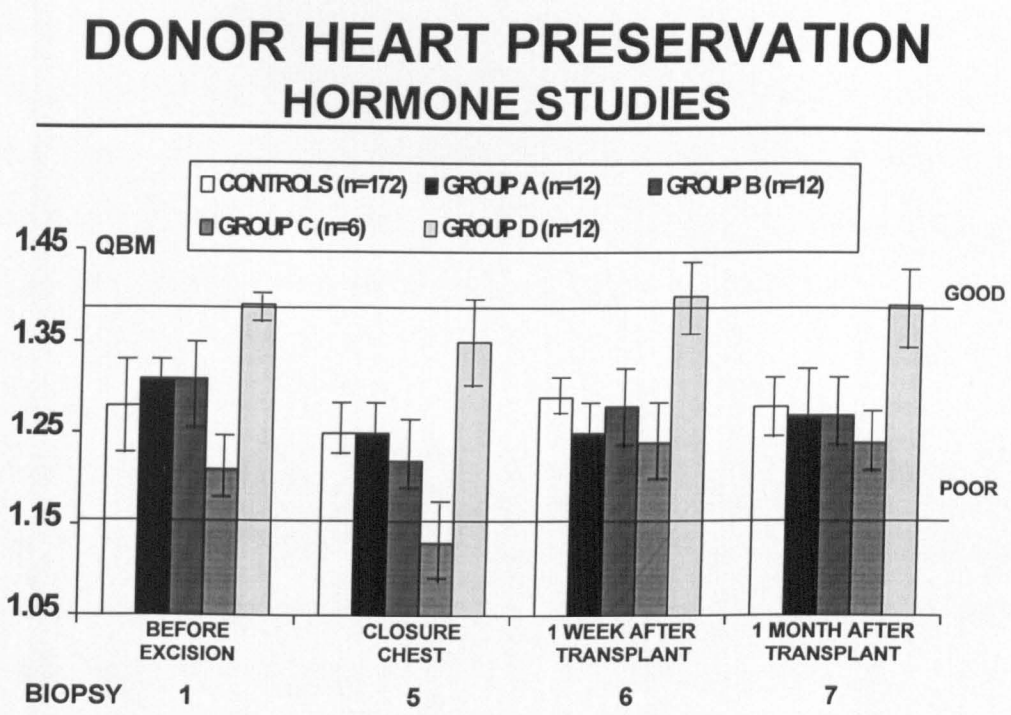


Figure 2B

Histogram showing the changes in QBM with time, following transplantation. The four groups, **A** - Group Controls, **B** - Bolus 2 μg, **C** - Bolus 4 μg, **D** - Infusion, are compared with the historical total group of hearts retrieved before the donor management regime was instituted. There were no significant differences between the group controls and the historical controls. The high dose T3 group (**C**) seemed to make a partial recovery within 1 week of transplantation. The peri-operative improvement seen in the Infusion group (**D**) is maintained up to at least 1 month post transplant, demonstrating that the benefit is sustained.

Early Mortality

There were 5 early deaths in this group of 42 patients, none of which could be directly attributed to donor organ dysfunction.

Group A	1	Acute Rejection
Group B	1	Acute Rejection
Group C	1	Acute Rejection
Group D	1	Acute Rejection
	1	Haemorrhage

QBM indicated that myocardial function in the ten surviving recipients of hearts in Group D remained better up to one month after transplantation than that of the other 3 groups (mean value 1.39 ± 0.05 compared with 1.27 ± 0.05, 1.27 ± 0.04 and 1.24 ± 0.03 for groups A, B and C respectively). Moreover, QBM in these hearts achieved the values of normal myocardium which were defined in earlier studies[76,77], as opposed to the preceding groups which never attained normal QBM.

Conclusions

This series of pilot studies indicated that the use of an *infusion* of T_3 was beneficial. The use of a single bolus of T_3 (Group B) was not associated with any discernible benefit, whilst the use of multiple bolus injections (Group C) may well have been harmful (decreased QBM post storage). However, it would seem that there is a clear benefit from the infusion regime employed in Group D.

The experience gained during the conduct of these studies, also made it apparent that in order to manage these patients optimally, it was necessary to institute comprehensive haemodynamic monitoring. It was therefore decided to source

suitable portable haemodynamic monitoring equipment and to add a cardiac trained anaesthetist to the Donor Team, whose responsibility it would be to take over donor management during the retrieval procedure. It also became clear that whilst haemodynamic assessment seemed impractical at first, this could be readily accomplished, given the experience gained. This was further enhanced by the development of a simple functional guideline in collaboration with the author's colleague, Charles Potter (Bibliography F).

2.4 THE MANAGEMENT REGIME

In reviewing the above experience and the literature, at this stage, the donor management strategy outlined below was formulated. Having initiated this regime it was decided to review the data at intervals of approximately every 50 donor procedures.

There are four main problem areas which need to be addressed in caring for the brain-dead donor:

1 Cardiovascular Dysfunction

This may be due to hypotension secondary to volume depletion, loss of vasomotor tone, endocrine depletion, hypoxia, electrolyte imbalances or direct myocardial dysfunction.

2 Respiratory Dysfunction

Pneumonia, aspiration, pneumothoraces, pulmonary oedema and pulmonary collapse commonly occur in donors, and may all contribute to a deterioration in respiratory function with subsequent hypoxaemia.

3 *Endocrine Dysfunction*

Impaired thyroid hormone release has been discussed above and posterior pituitary dysfunction has been recognised to be common[57] in brain dead donors. The absence of vasomotor impulses in brain death seem to result in a dependency on ADH, which has synergistic action with adrenaline[49]. Insulin deficiency is also common with resultant diabetes insipidus and subsequent large urinary volume and electrolyte losses[80]. There may also be an associated limitation of intracellular glucose as a substrate.

4 *Hypothermia*

Brain death produces a loss of hypothalamic temperature control, effectively rendering the donor poikilothermic. Active warming is required to maintain core temperatures above 35°C[81].

Strategy:

A. Ventilate to normocarbia (4.5 - 5.5 kPa) using large tidal volumes (15 ml/kg) with either a low respiratory rate or deadspace.

B. Use a minimal inspired oxygen consistent with adequate oxygen delivery (arterial saturation > 98%).

C. Ensure clear airway using tracheal suction and check position of endotracheal tube. Check for bilateral air entry.

D. Prepare following access IV lines:

i) Left arterial (radial or brachial).

ii) Right internal jugular triple lumen.

iii) Right internal jugular Swan-Ganz introducer.

E. Use the above lines in the following manner:

i) Swan Introducer - Blood products and colloids.

ii) Small triple lumen line - ADH/Adrenaline infusion.

iii) Middle triple lumen line - T_3 and insulin infusions.

iv) Large triple lumen line - Inotropes and dilators.

Use warming coil for all administered fluids.

F. Target the following status with reference to the Nomogram (Bibliography F):

i) Cardiac Index > 2.6 $l.min^{-1}.m^{-2}$

ii) SVR 800 - 1200 $dynes.sec^{-1}.cm^{-5}$

iii) LV Stroke Work Index > 20 $g.m.m^{-2}$

iv) At minimum preload (< 10 mmHg)

G. Measure baseline haemodynamics and if in HRT category proceed as below:

H. Use ADH/Adrenaline to increase afterload (max. 5 IU/hr)

Use Sodium Nitroprusside to decrease afterload (max 4 $\mu g.kg^{-1}.min^{-1}$).

Use Dopamine if all else fails to produce an adequate output.

I. Use Dextrose/Saline/Potassium to replace urinary losses.

Use colloid to increase preload.

Use whole blood or packed cells to keep HCT 30 - 35%.

J. Measure blood glucose and infuse Insulin on the following basis:

Blood Sugar	Insulin Infusion
> 15 mmols	5.5 IU/hr
8 - 15 mmols	3.5 IU/hr
4 - 8 mmols	1.5 IU/hr
< 4 mmols	GIK 1.5 $ml.kg^{-1}.hr^{-1}$

K. Triiodothyronine - Give bolus of 4 μg followed by an infusion of 3 μg/hr.

L. Vasopressin - Give 1 Unit followed by an infusion of 1 U/hr. Titrate to SVR up to a maximum of 5 U/hr. (Use Nomogram for guidance - Bibliography F).

2.5 ASSESSMENT

It was decided to divide all potential donors into three groups based on initial functional evaluation. Those which had normal function would be managed without any hormone supplements. Those donors showing marginal function would receive the HRT as outlined above. Those donors showing markedly impaired function would receive both HRT and be placed on supportive bypass (CPS), if they met the additional criteria for this group; age < 45 years, no sepsis and no obvious cardiac disease. The choice of haemodynamic limits for each classification was based on experience in the field of mechanically assisted circulation[82]. The details and results of this approach are presented in Chapter 3.

2.6 DISCUSSION

The most common cause of brain death in human cardiac donors is head trauma, with subsequent intracranial haemorrhage and diffuse swelling. An intracranial

vascular accident, such as haemorrhage or ischaemic stroke, is the next most common event. In both these cases, there is an increasing mass effect in the brain, compression of brain tissue and subsequent venous congestion, increasing turgor, transentorial herniation, brain stem compression and subsequent infarction. Clinical brain death is diagnosed, following the establishment of specific pre-conditions, and is now well established in the western world as being synonymous with the death of the individual in that the condition is irreversible[83].

The deleterious effects of catecholamines on the heart have been widely recognised for a long time as have the cardiotoxic effects of amines. Overproduction of endogenous catecholamines derived from adrenal pheochromocytomas[84], tetanus toxin induced overstimulation[85], raised intracranial pressure[86], cocaine abuse[87] and the effects of drowning[88] may all produce myocyte necrosis. There is also evidence that coronary vasospasm is associated with catecholamine excess leading to acute myocardial ischaemia[89].

It is therefore not surprising that the early descriptions of an association between neurological injury and myocardial damage would have implications for those engaged in heart transplantation. In 1954 Smith and Tomlinson[44] noted subendocardial haemorrhage in the myocardium of patients dying from intracranial lesions. Burch and co-workers[90] reported specific electrocardiographic abnormalities simulating myocardial ischaemia in patients following intracranial haemorrhage, traumatic brain injury, grand mal seizures, encephalitis, brain tumour and meningitis. They pointed out that, "It is reasonable to assume that a heart removed from a donor with extensive brain damage is abnormal and that such injury would be superimposed upon whatever structural and biochemical abnormalities occurred

during the terminal illness" e.g., as a result of hypoxia, electrolyte disorders, acid-base imbalance, administration of catecholamines etc[72]. Greenshoot and Reichenbach[39], after studying the myocardium of patients dying from subarachnoid haemorrhage, reported histological evidence of myocardial injury in all cases. Experimentally they showed that stimulation of the midbrain reticular formation produced ECG changes and myocardial injury in cats, and as cells adjacent to intramyocardial nerves were worst damaged, they proposed that this necrosis was due to the release of catecholamines from the adrenergic nerve endings in the heart. Heggtveit in 1970[91] studied the myocardium of 100 patients dying from cerebral haemorrhage or head injury, having excluded those with evidence of significant thoracic injury, shock or coronary disease, and found that "one half of the hearts exhibited gross subendocardial haemorrhages". He warned that in the selection of donor hearts, the presence and severity of 'neurogenic heart lesions' should be assessed as far as possible "since such occult cardiac damage may conceivably contribute to the failure of some transplants and obscure or complicate the histological manifestation of rejection in others". In 1986 Novitzky et al[45] reported that if the hearts of baboons were denervated prior to the induction of brain death, myocardial injury did not occur. They suggested that endogenous catecholamines released during the process of dying resulted in increased calcium uptake by the cells, thus inducing myocyte necrosis; they also postulated that such damage occurring in donor hearts might result in early failure after transplantation.

Electrocardiographic assessment forms part of the screening of donors prior to organ donation. Yet in spite of satisfactory ECGs, 42% of the 172 donor hearts in the original study[79] had significant myocardial injury as assessed by QBM in biopsies taken shortly before excision. This was not surprising since Samuels[41] reviewing the

problems of neurogenic heart disease in 1987 stated that "electrocardiographic abnormalities improve, often dramatically so, with brain death", thus underlining the unreliable nature of ECG analysis in the donor. Blum[58] induced angina-like ECG disturbances following hypothalamic stimulation in non-brain dead cats and squirrel monkeys and reported that myocardial necrotic and haemorrhagic pathology evolved with recurrence or prolonged duration of the stimulus. He observed that in both patients and experimental animals there were great individual and significant interspecies differences in response to the CNS stimulus.

In the earlier study of myocardial preservation during transplantation it was not possible to determine whether the poor myocardial function detected by QBM in a proportion of the donor hearts before excision was mediated by cerebral damage or whether it resulted from inadequate supportive measures after brain death. More recent work by Flamang's group shows that the important feature of brain death is the rate of intracranial pressure rise, prior to brain death. The more acute, the greater the catecholamine release and the more severe is the resultant impairment of myocardial function[59]. Given the lack of evidence for this damage on post transplant biopsies, it seems likely that this damage can be resolved with time. It may be that the severely damaged hearts self select themselves before donation takes place, or particular management regimes may help to resolve the damage.

All these studies have therefore suggested that the success of a transplant operation could be improved by some form of donor pre-treatment to prevent or reverse the harmful effects of brain death. Novitzky and Cooper observed significant reductions in the circulating levels of insulin, glucagon, thyroxine and cortisol in baboons and pigs following brain death and increased anaerobic metabolism[42,55,64,85]. Following

replenishment of these hormones T_3 levels normalised and there was a return to aerobic metabolism and myocardial function improved. In 21 donors pre-treated and monitored for 3-8 hours before retrieval they recorded improved ECGs and a significant reduction in their need for inotropic support[55]. In a subsequent study of 70 donors[85] they reported that hormonal pre-treatment allowed them to salvage some donor hearts which would previously have been considered unsuitable and that the haemodynamic stability resulting from this therapy allowed them to maintain 5 donors overnight and transplant the organs the following morning. There were no deaths from low output failure amongst recipients of these hearts.

Various groups[68-77] have verified the fact that, following brain death, serum T_3 levels are often abnormal. Koller[68] studied 82 patients treated in ICU for head injury and noted that their T_3 levels were in the lower normal range. These levels remained stable in the 46 who survived, but fell to subnormal levels in the remainder by the time of organ retrieval. Howlett[92] found subnormal T_3 levels in 81% of donors studied at the time of organ donation, which was comparable to the 86% who had abnormal values in the studies reported above. Some groups have attempted to correlate donor T_3 levels with the need for inotropic support of the heart before and after transplantation. Gifford[67] found no differences in this respect when comparing hearts from donors with subnormal versus undetectable levels of T_3. In contrast Wahlers[64] compared hearts from donors with normal and subnormal T_3 levels and found that the dosage and duration of inotropic support was significantly greater in the latter following transplantation. Montero[71] found abnormal T_3 levels in 86% of donors and then did ultrastructural studies measuring the number of T_3 receptors present in the myocardium. He found that patients with a low serum T_3 had a low number of occupied T_3 nuclear receptors and that the degree of myocardial damage

correlated with the reduction in occupied receptors. None of these studies tested the effect of the replacement therapy advocated by Novitzky and colleagues.

More recently it has become recognised that chemical hypothyroidism is relatively common in critically ill patients[68] and that this can result in profound left ventricular dysfunction, particularly when in association with myocardial ischaemia[70]. This finding has been underlined by the observation that the *combination* of cerebral injury and subsequent donor organ preservation ischaemia has a harmful effect on donor kidneys[93] and hearts[61], particularly with respect to right heart function[94].

Although there was a reduction in the need for inotropic support in each of the three groups of donors following pre-treatment with T_3 in the above pilot studies, the requirement for inotropic support before and after transplantation did not correlate with donor serum levels. However, this treatment regime, whilst following the dosage recommended by Novitsky et al, was only instituted for 1 - 3 hours, due to local constraints, compared with 3 - 8 hours accomplished by Novitsky's group.

Macoviack[71] in discussing whether thyroid hormone replacement was beneficial concluded that "there appears to be no rationale for giving human cardiac allograft donors any form of thyroid hormone replacement when careful fluid, electrolyte and glucose balance is maintained in combination with generally meticulous donor management". However the results of his own study of 22 donors compared poorly with that of Novitzky and Cooper[61] since after a period of "meticulous management" 2 hearts became unsuitable for transplantation and 2 recipients died from cardiac failure (one due to recipient pulmonary hypertension). Novitzky's control study of 26 carefully managed donors had yielded similar results to those of Macoviack, but the

superior clinical outcome of the next 70 hearts in which he incorporated hormonal pre-treatment into pre-transplant care indicated that it *did* confer an additional benefit.

The reasoning behind switching to an infusion was both as a result of Novitsky's observation that an increased dose was sometimes required to obtain a therapeutic effect, and an attempt to stabilise serum levels. QBM indicated that donors receiving the infusion regime exhibited good myocardial function at excision and that these hearts remained better than those in the other groups throughout transplantation. This improved function was not a temporary response induced by any inotropic effect of T_3, since QBM in endomyocardial biopsies taken one week and one month after transplantation remained good. The fact that only one of these hearts had needed support after implantation confirmed the biopsy assessment of good myocardial function in this group of hearts.

2.7 CONCLUSION

In reviewing the results of the above studies and the literature, there would seem to be a clear case for using T_3 in those donors who demonstrate impaired function. The addition of ADH and insulin is more speculative but would seem to be justified in the light of the available evidence.

The hypothesis was therefore that the use of a standardised donor management strategy, based on objective functional measurements, would allow a safe increase in the numbers of donor hearts retrieved *and* in the post transplant functional quality of these hearts. The realisation of this goal is described in the next chapter (Chapter 3).

CHAPTER 3

DONOR HEART RESUSCITATION STUDIES

3.1 BACKGROUND

Transplant medicine has enjoyed a high profile image since its inception, but this has largely been focused on the drama of the surgery and the intellectual challenges imposed by the necessity of immunological manipulation. Donor management on the other hand, is probably the most neglected area of transplant medicine. During the pilot studies into possible methods of reversing the adverse metabolic effects of brain death, it became clear that an overall systematic approach was required and that central to this was objective patient monitoring. The literature suggests that failure to provide adequate physiological support to potential donors accounts for at least 25% of lost donor organs[72].

New road safety laws and the compulsory wearing of seat belts, have had, and continue to have, an impact on the number of solid organ donors[95] (Figure 3A) in most western countries. In some countries, including the UK, there is an increasing under-resourcing of intensive care beds, which probably also has an impact on cadaveric organ referral [96].

Early graft failure accounts for approximately 34% of acute deaths in heart transplant recipients[19]. In addition there is undoubtedly a further significant number of patients

who are compromised by poor graft function, related to inadequate donor management and/or preservation.

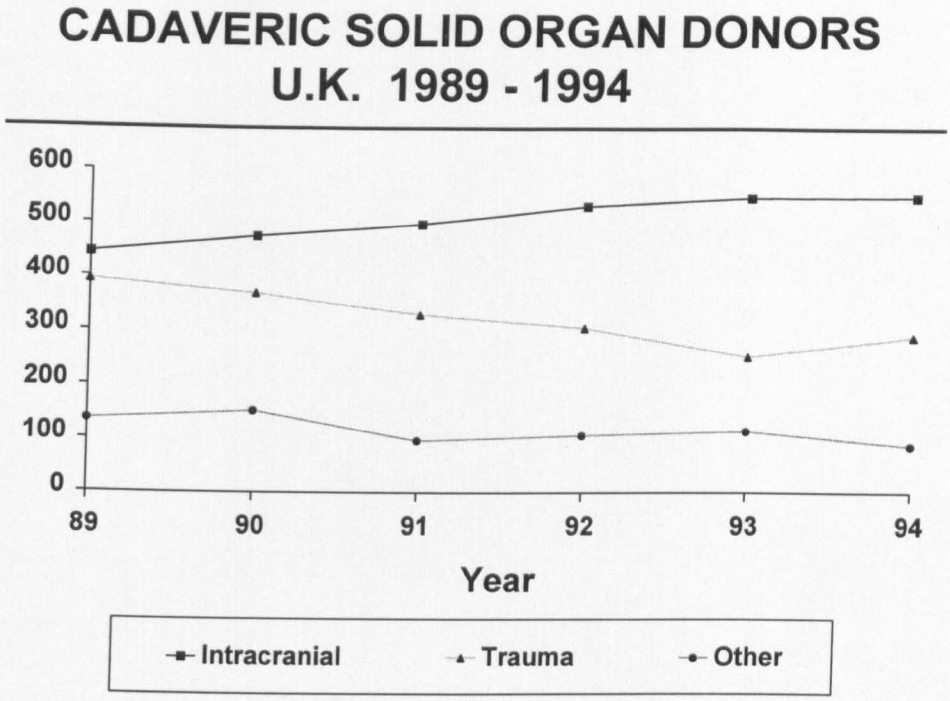


Figure 3A Changes in Causes of Death for Cadaveric organ donors in the UK, showing the downward trend in donors from traumatic deaths (mainly road traffic accidents), over the past 5 years. There has been a partial corresponding increase in donors from Intracranial haemorrhage, over the same time span, but an overall reduction in total numbers.
Source: UKTSSA.

Prompted by this increasing shortfall of donor organs, several centres have reported extensions to the "classical" donor criteria with respect to age[97], ischaemic time[98], inotropic support[99], adverse haemodynamics[100], size mis-matching[101], cause of death[102-104] and infection[105]. Whilst some of these experiments have been successful in individual cases, most have not, especially when more than one contraindication is present[106].

The aim of the work described in this chapter was to explore the hypothesis advanced in Chapter 2; that the quantity and quality of retrieved donor hearts could be substantially improved by adopting a strategy of donor management based on objective functional monitoring together with specific standardised interventions.

3.2 MATERIALS AND METHODS

Between October 1990 and October 1993, 150 multi-organ donors were fully instrumented during the retrieval operation[107]. This involved transport to the donor hospital of specially sourced compact monitoring equipment and the institution of invasive arterial, internal jugular venous, and Swan-Ganz pulmonary artery catheterisation, as soon as the donor arrived in the operating room. Ventilation, fluid replacement and electrolytes were optimised and the first set of measurements performed. Further optimisation[73] was carried out whilst the cardiac surgeon performed a median sternotomy and visually examined the thoracic organs.

During this inspection a comprehensive set of measurements was performed. Classification of the donor was carried out according to the criteria in Table 3.1. Those donors judged to be *Marginal* were started on the HRT regime described in Chapter 2. Those donors who fell into the Cardio-pulmonary Support (CPS) category were electively placed on support bypass in addition to receiving HRT (Bibliography E). The basis for this classification is discussed in Chapter 2.

TABLE 3.1

CLASSIFICATION OF POTENTIAL CARDIAC DONORS INTO THREE FUNCTIONAL GROUPS BASED ON HAEMODYNAMIC PARAMETERS

MEASUREMENT	STANDARD	HRT	CPS
CARDIAC INDEX ($l.min^{-1}.m^{-2}$)	> 2.6	< 2.5	<2.0
CVP (mmHg)	< 10	> 10	> 15
PCWP (mmHg)	< 10	> 10	> 15
LV POWER (Watts)	> 0.6	< 0.6	< 0.4
or LV Stroke Work Index $g.m.m^{-2}$	> 20	< 20	< 15
INOTROPES ($\mu g.kg^{-1}.min^{-1}$)	NIL	> 1.0	> 8.0

CVP = Central Venous Pressure
PCWP = Pulmonary Capillary Wedge Pressure
LV Power = Left Ventricular Static Power
Inotropes = Dopamine or Dobutamine

During the splanchnic dissection further sets of measurements were taken, and these were used in conjunction with the Functional Nomogram (Bibliography F) as a guide to management. Once the splanchnic dissection was completed, and with the cardiac surgeon back at the table, the final set of measurements was taken as a basis for donor organ acceptance (Table 3.2).

TABLE 3.2

TRANSPLANT ACCEPTANCE GUIDELINES

Mean Arterial Pressure	> 60 mmHg
Central Venous Pressure	< 12 mmHg
Pulmonary Capillary Wedge Pressure	< 12 mmHg
Left Ventricular Stroke Work Index	> 15 $g.m.\ m^{-2}$
Inotropes	< 5 $\mu g.kg^{-1}.min^{-1}$

These minimum acceptance criteria are based on studies of survival of patients in cardiogenic shock[108,109] and those used as entry criteria for patients requiring mechanical ventricular assist devices[82].

In order to provide some idea of major events which take place during a multiorgan retrieval operation, the following summary outlines the activities of the Thoracic and Abdominal Teams. The timing is related to a hypothetical operation starting at 8 am.

TABLE 3.3

SUMMARY OF MAJOR EVENTS DURING MULTI-ORGAN RETRIEVAL

TIME	THORACIC TEAM	ABDOMINAL TEAM
0800h	Team arrives-contacts base hospital	
0815h	ICU assessment and transport of donor to theatre	
0840h	Placement of lines/Swan Ganz Catheter	
0900h	Sternotomy-Inspection of Organs Optimisation-Baseline measurements	Team arrives
0920h	Bronchoscopy/Lavage	Dissection starts
1040h	Heparinisation	Dissection complete
1050h	Placement of Pulmonary artery Cannula Innominate and Tracheal dissection Prostacylin infusion starts	
1100h	Placement of cardioplegia cannula	
1110h		Cannulation of abdominal Aorta, Portal Vein, IVC
1120h	Division of SVC/Clamp IVC Clamp Asc. Aorta/run Cardioplegia Topical cold	Run Aortic and Portal perfusions. Vent IVC
1125h	Run pulmonary infusion Topical cold	Complete dissection of Abdominal organs
1130h	Complete dissection of H-L block	
1135h	Move organs to back table	
1140h	Inspect for congenital defects Staple trachea	
1145h	Divide organs and package	Move liver to back bench Coeliac perfusion
1155h	Organs boxed for transport	Kidneys on back bench and perfused
1200h		Organs packaged

3.3 RESULTS

Of the 150 multi-organ donors, there were 133 which yielded transplantable thoracic organs; 87 heart and 46 heart and lung blocks, together with splanchnic organs. Seventeen donors were not used; 9 on the grounds of dysfunction which was refractory to all of our resuscitative efforts, and 8 on the grounds of cardiac disease (Table 3.5).

There were 52/150 donors which fell *well outside* (Table 3.4) our acceptance guidelines once comprehensive monitoring was in place and the data available (Table 3.2). On initial inspection 33/52 of these were identified; 21 on the grounds of low mean arterial pressure (mean 47 mmHg), 10 on the grounds of a high CVP (mean 18 mmHg) and 2 requiring high inotropic support (25 $\mu g.kg^{-1}.min^{-1}$). Figure 3B illustrates the individual and mean values at the start and end of the procedure for these groups, compared with the mean values for the remaining donors.

One donor was not used because of palpable coronary disease and one because of refractory dysfunction.

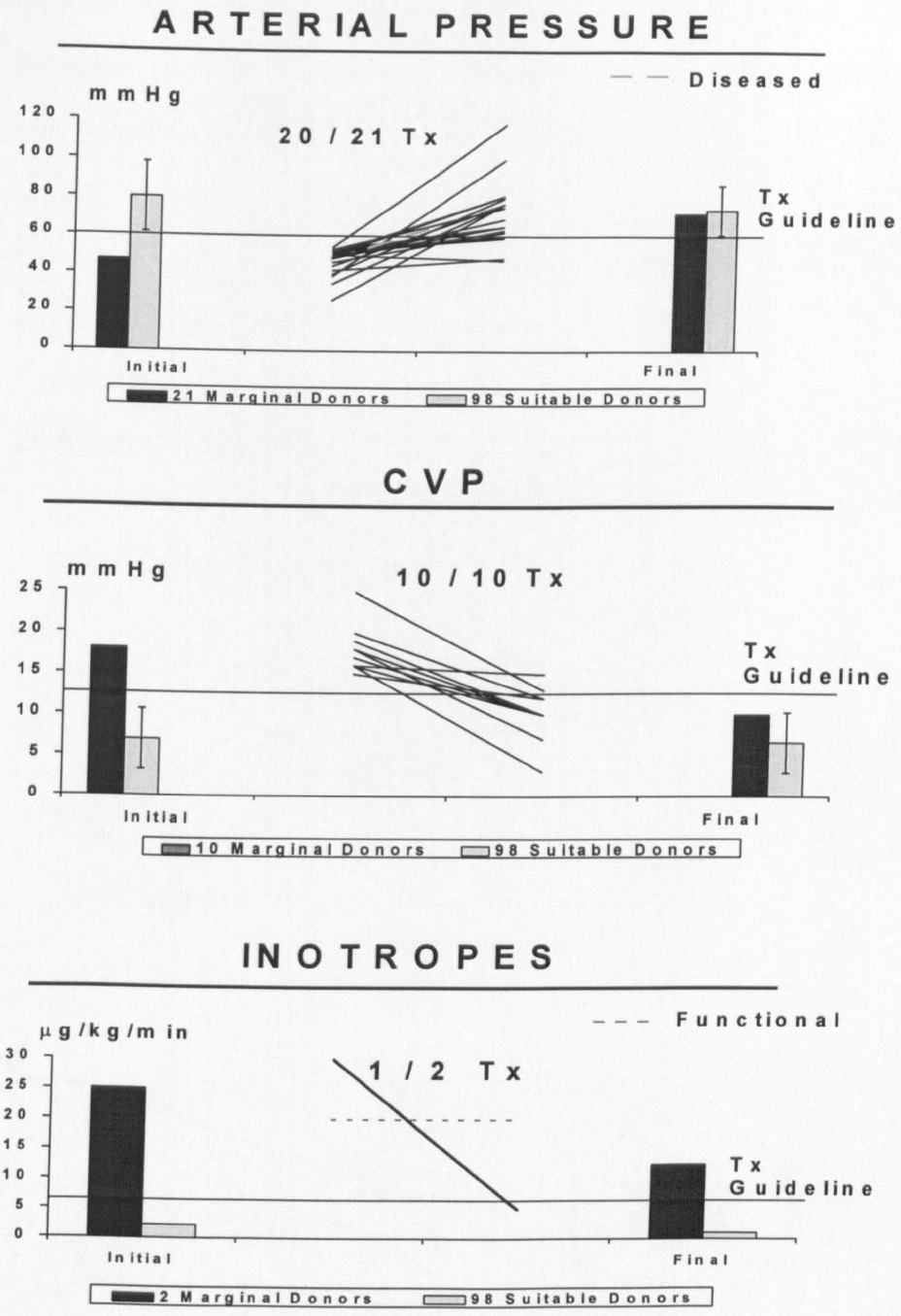


Figure 3B Graphical representations of the peri-operative changes in those parameters measured without the need of invasive monitoring and which formed the basis of classification into *Suitable* and *Marginal* groups. The horizontal line represents the minimal acceptable value for transplant acceptance, in each case.

With full monitoring there were a further 19/52 who fell *well outside* our acceptance guidelines; 13 on the grounds of high pulmonary capillary wedge pressure (mean 19.8 mmHg), and 6 on the grounds of a low stroke work index (12.8 g.m. m^{-2}). Figure 3C illustrates the individual and mean values at the start and end of the procedure for these groups, compared with the mean values for the remaining donors. In the 13 patients with high PCWPs there was also a significant left/right imbalance; mean CVP 10.2 mmHg with a mean PCWP of 19.8 mmHg, which would not have been detected without full monitoring.

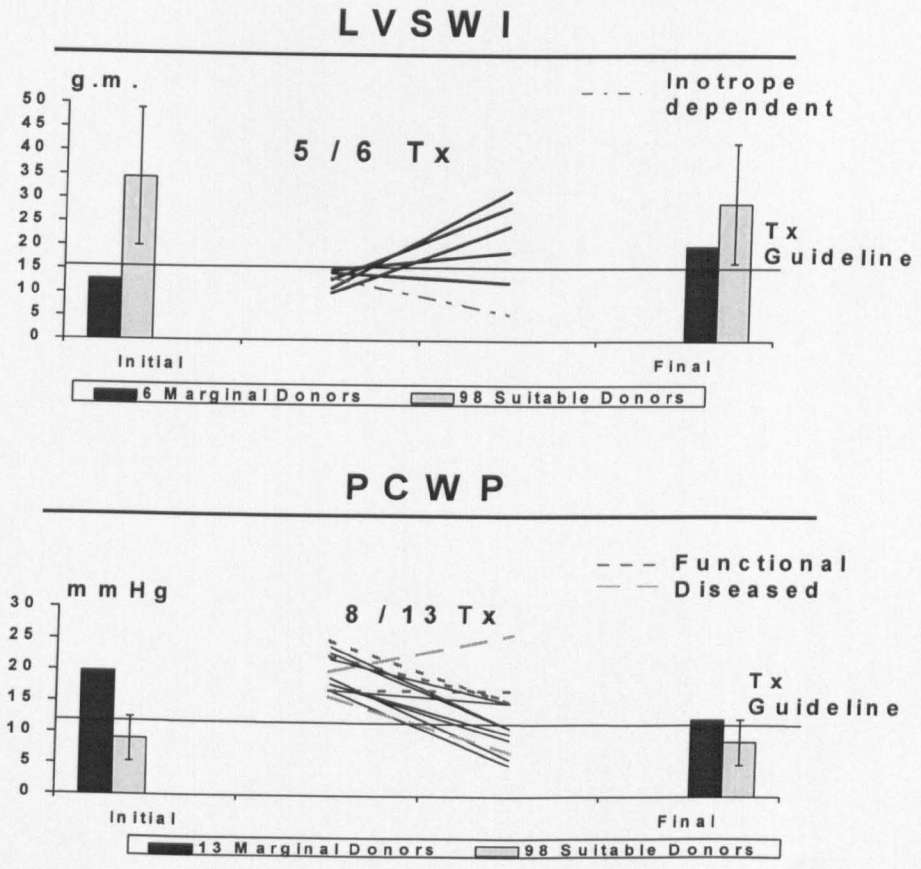


Figure 3C Graphical representations of the peri-operative changes in those parameters measured with the use of invasive monitoring and which formed the basis of classification into *Suitable* and *Marginal* groups. The horizontal line represents the minimal acceptable value for transplant acceptance, in each case.

Six of these donor hearts were not used; 2 because of coronary disease, 1 because of left ventricular hypertrophy and 3 because of refractory dysfunction.

Forty four of the 52 marginal donor organs were successfully transplanted. Of the 8 hearts not transplanted; 4/8 were inotrope dependent and the remaining 4 had evidence of cardiac disease (coronary disease 3, left ventricular hypertrophy 1) despite acceptable function (Table 3.5). Table 3.4 gives the comprehensive data set for the 52 initially *Marginal* donors.

TABLE 3.4

Comprehensive data on the 52 initially marginal donors

Pt Num	AoP mmHg	CVP mmHg	INOTROPES	PCWP mmHg	LVSWI $g.m^{-1}$	NOT USED	DEATHS
1	52	8	0	10	12.8		CVA Day 16
2	51	6	0	9	15.5		
3	53	5	0	9	18.7		
4	52	1	0	4	15.1		
5	53	1	5	4	36.7		
6	35	6	5	7	13.8		
7	39	6	0	7	13.9		
8	48	6	2.5	10	19.6	LVH	
9	50	15	5	17	6.1		Infection Day 16
10	50	2	0	5	6.5		
11	42	5	14	3	14.9		
12	40	0	4	2	20.1		
13	44	0	12	7	16.2		
14	49	3	5	1	21.2		
15	54	12	2.5	8	18.9		
16	51	4	3.0	8	35.6		
17	53	9	5	11	18.9		
18	46	1	5.5	2	18.9		
19	48	0	0	3	33.6		
20	50	4	2.5	6	22.5		
21	26	1	15	7	7.9		
22	85	15	0	15	50.0		
23	60	16	8	19	22.1		Tamponade 3 mths
24	81	17	6.5	20	39.4		
25	62	18	5	23	15.6		Infection 14 mths
26	114	16	0	20	53.4		
27	60	25	5	11	19.0		
28	79	19	8	19	27.5		
29	62	20	6	20	28.9		
30	65	16	16	9	18.9		
31	112	18	4	24	49.2		
32	56	9	20	7	27.2	Inotrope dep	
33	60	1	30	11	67.1		
34	55	7	0	22	20.2		
35	140	11	0	17	50.3		
36	71	13	6	20	16.3	LVH	
37	80	13	0	19	34.8		
38	117	9	0	17	96.6		
39	86	10	0	18	24.3		
40	92	10	5	23	42.3		ARDS Day 1
41	65	9	10	24	26.1		
42	76	11	12	17	26.3	Inotrope dep	
43	75	10	6	16	19.5	CAD	
44	66	14	7	22	38.4	CAD	
45	81	8	2.5	25	15.7	Inotrope dep	
46	84	6	0	18	21.5		
47	63	7	20	20	10.1		Arrythmia 12 days
48	62	6	3	13	14.4		
49	65	10	2.5	11	11.0		
50	72	10	0	11	13.5	Inotrope dep	
51	78	3	2.5	6	13.3		
52	62	12	0	14	14.9		

Shading indicates basis for classification: LVH = Left Ventricular Hypertrophy
CVA = Cardio Vascular Accident
CAD = Coronary Artery Disease
ARDS = Adult Respiratory Distress Syndrome

Figure 3D illustrates the changes in inotrope support for the entire group, together with each of the marginal groups. The horizontal lines provide reference points from the initially *Suitable* group. Overall, 63% of donors initially required inotropic support with a mean dose of 6.8 $\mu g.kg^{-1}.min^{-1}$, which reduced to 45% requiring a mean dose of 4.8 $\mu g.kg^{-1}.min^{-1}$, at the end of the retrieval procedure. (Dopamine and Dobutamine were the only inotropes used, and distinctions between the two were not made, for the purposes of this study.)

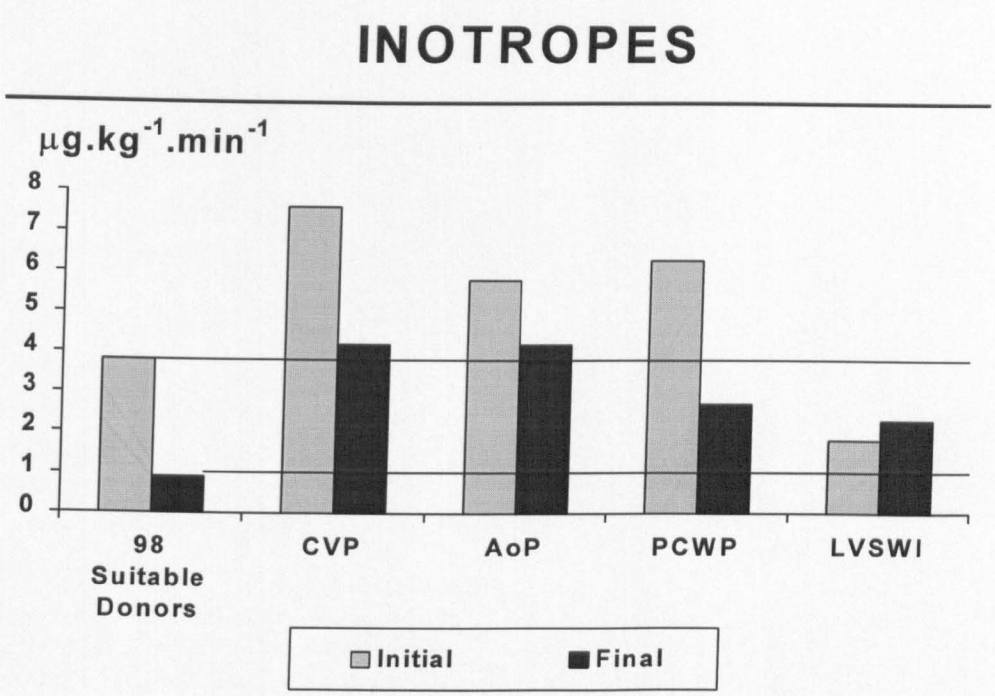


Figure 3D

Graphical representations of the peri-operative changes in the concommitant requirements for inotropes in the major classification groups. This illustrates that in all but the LVSWI group there was an improvement in function in the face of *reduced* inotropic drive.

The horizontal lines provide reference points from the initially *Suitable* group.

Categories refer to the *primary* grounds for classification as *Unsuitable.*

Of the 150 donors, 98 were within or close to our haemodynamic criteria on inspection. Nine were not transplanted; 4 on the grounds of significant coronary artery disease, 3 became inotrope dependent, 1 developed right heart failure during the procedure and 1 was poisoned with an overdose of lignocaine. This means that only 5% overall, were refractory to the management regime.

TABLE 3.5

Donor Outcomes

	Inspection	Monitoring	Tx	Not Tx		Total
				Disease	Function	
Meet Guidelines	117	98	89	4	5	9
		19				
Marginal	33	52	44	4	4	8
TOTALS	150	150	133	8	9	17

Transplanted Organs : 87 Hearts 46 Heart / Lung

This table illustrates the evolution of the two groups during the retrieval procedure:- 19 initially suitable donors moved into the marginal group when the results of invasive monitoring were available. There were 17 donor organs which were not transplanted, distributed as shown, some because of cardiac disease which became apparent at operation and some because of ongoing cardiac dysfunction.

Overall there were 133 donors yielding transplantable hearts (and 76 lungs). The mean haemodynamic data is shown in Table 3.6. *Initial* values are those obtained following placement of a Swan Ganz catheter at the beginning of the procedure and *Final* values refer to those obtained following HRT therapy and immediately prior to retrieval. Values showing statistically significant changes are indicated with the

relevant p value (paired T test). The table illustrates very clearly that not only is there a general improvement in haemodynamics, but that there is also a significant reduction in standard deviation across the measured parameters and which reaches statistical significance in five of these (F test).

TABLE 3.6

DONORS YIELDING TRANSPLANTABLE HEARTS

PARAMETER		INITIAL	(SD)	FINAL	(SD)
Heart rate	bpm	109	(19.2)	106	(18.2)
AoP	mmHg	74.8	(21.3)	74.8	(13.4)***
CVP	mmHg	7.5	(4.7)	6.9	(4.0)
PAP	mmHg	18.2	(6.5)	15.4***	(5.0)*
PCWP	mmHg	9.9	(5.3)	8.9*	(3.9)**
CO	l/min	6.9	(2.7)	6.0**	(2.3)
CI	$l.min^{-1}.m^{-2}$	3.9	(1.6)	3.4**	(1.3)
SVR	$dynes.s.cm^{-5}$	881	(401)	1016**	(410)
PVR	$dynes.s.cm^{-5}$	104	(58.4)	92	(48.9)
LVSWI	$g.m.m^{-2}$	31.7	(15.9)	29.4	(12.8)*
RVSWI	$g.m.m^{-2}$	4.2	(2.8)	3.0**	(2.1)***
LVPo	Watts	1.0	(0.5)	0.9	(0.4)
SI	$ml.bt^{-1}.m^{-2}$	35.7	(12.8)	32.8	(2.4)
Inotrope	$\mu g.kg^{-1}.min^{-1}$	6.8		4.8	(see text)

* = p<0.01 ** = p<0.001 *** = p<0.0001

A0P = Arterial pressure
CVP = Central Venous Pressure
PAP = Pulmonary Artery Pressure
PCWP = Pulmonary Capillary Wedge Pressure
CO = Cardiac Output
CI = Cardiac Index
SVR = Systemic Vascular Resistance
PVR = Pulmonary Vascular Resistance
LVSWI = Left Ventricular Stroke Work Index
RVSWI = Right Ventricular Stroke Work Index
LVPo = Left Ventricular Static Power
SI = Stroke Index.

Outcomes

Initially Suitable Donors

The overall survival in the group of 89 recipients who received organs from initially *Suitable* donors is shown below.

TABLE 3.7

SURVIVAL

SUITABLE DONORS	
68/89 (76%) Alive and Well	13-48 months post transplant
11/89 (12%) 30 Day Mortality	
3 Elevated PVR	Heart
3 Graft Failure	1 Heart, 2 Heart/Lung
1 Pancreatitis	Heart/Lung
1 Infection	Heart/Lung
1 CVA	Heart
1 Arrythmia	Heart
1 Pulmonary Embolus	Heart
10/89 (11%) Late Deaths	
2 CVA 7 & 25 months	Heart & Heart/Lung
1 Pancreatitis 3 months	Heart
4 Coronary Disease 5,7,9,16 months	Heart
1 Malignancy 4 months	Heart
1 Multi-organ failure 3 months	Heart/Lung
1 Infection 4 months	Heart/Lung

Initially Marginal Donors

The overall survival in the group of 44 recipients who received hearts from *Marginal* donors is shown below.

TABLE 3.8

SURVIVAL

MARGINAL DONORS	44/52 Transplanted
37/44 (84%) Alive and Well	13-48 months post transplant
5/44 (11%) 30 day mortality	
Arrythmia	Heart
Infection	Heart
Acute Respiratory Distress	Heart
Cerebrovascular Event	Heart and Lung
Infection	Heart/Lung and Liver
2/44 (5%) Late Deaths	
Tamponade 3 months	Heart
Infection 14 months	Heart and Lung

As is evident from the above, only one of these deaths could be directly attributed to a cardiac cause. These results compare well with the International Registry 30 day mortality figures of 9.8% (hearts) and 21% (heart and lung) and 1 year Registry mortality figures of 78% (hearts) and 60% (heart and lung)[19]. Actuarial survival curves show no difference between these two groups and the Registry survival curve, using data since 1985 (Figure 3E).

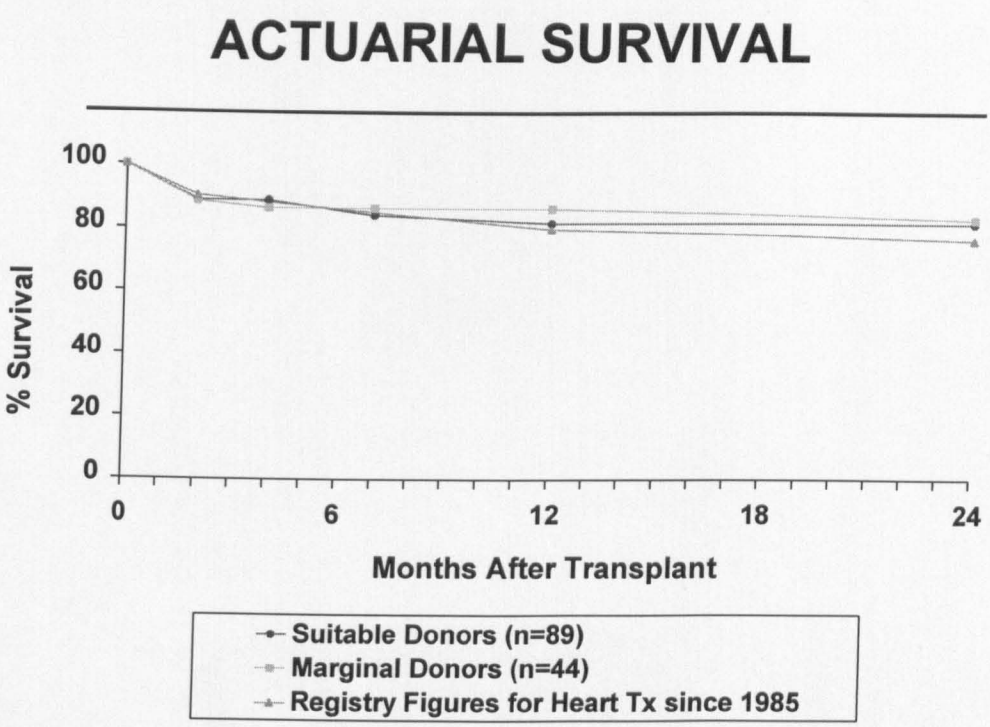


Figure 3E Survival curves show no differences, over the first two years post transplant, between the *Suitable* and *Marginal* groups and between these and the International Registry data covering the same time period.

Of the patients surviving to discharge there were 80 heart and 38 heart-lung recipients. Time to discharge was similar for the heart recipients irrespective of the donor group (25 days in the *Suitable* group compared with 23.6 days in the *Marginal* group). However, in the heart-lung recipients there was a significant difference (p = 0.03, 2-tailed T test) between the two groups in which those recipients receiving hearts from initially marginal donors had a shorter hospital stay (27.7 days) compared with those receiving hearts from suitable donors (36.4 days). Their mean length of hospital stay is shown in Figure 3F.

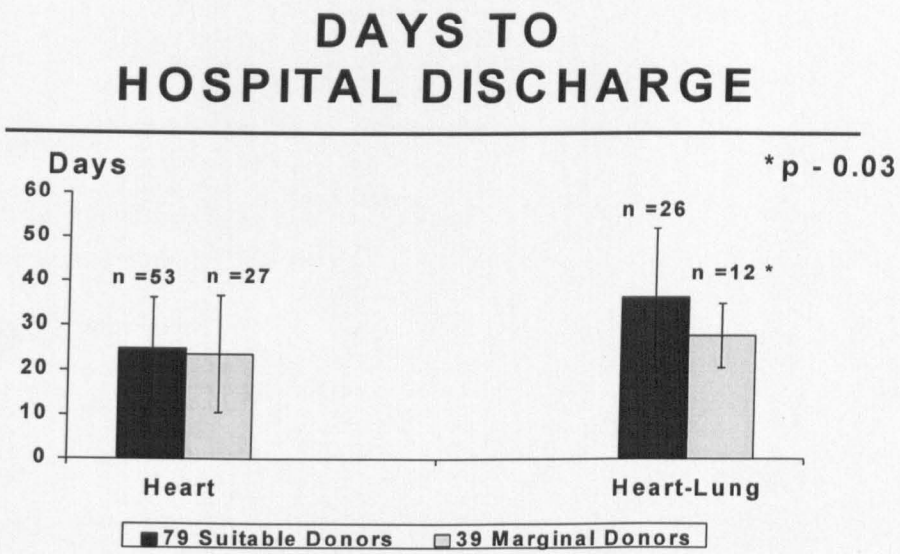


Figure 3F There were no differences in hospital stay between the two donor groups in Heart Transplant recipients, however those Heart-Lung recipients who received donor organs from the *Marginal* (managed) donors had a shorter and less complicated hospital stay. The reason for this is unclear.

There were, however some differences between the ***Heart*** and the ***Heart-Lung*** donors as illustrated in Table 3.9. These were; that heart donors were on average 3.5 years older than heart-lung donors, the heart donors were significantly bigger ($p<0.03$), and that there were 60% males in the heart donor group compared with only 48% in the heart-lung group. However, it is unclear why a smaller female donor should apparently respond better to our donor management regime.

TABLE 3.9

DEMOGRAPHIC DIFFERENCES BETWEEN HEART AND HEART-LUNG DONORS

	HEARTS	HEART & LUNGS
All Donors	87 (65%)	46 (35%)
Average Age (yrs)	34.1	30.6
Body Surface Area (m^2)	1.83	1.74
Male Donors	52 (60%)	22 (48%)
Average Age (yrs)	33.6	28.2
Body Surface Area ($m^{2)}$	1.92	1.82
Female Donors	35 (40%)	24 (52%)
Average Age (yrs)	35.0	32.8
Body Surface Area (m^2)	1.70	1.67

3.4 DISCUSSION

The donor management regime, described in this chapter, has been developed over the past four years, as detailed in Chapter 2. This was originally inspired by the Cape

Town group[79], who investigated the hormonal consequences of brain death with subsequent influence by publications from Japan, with respect to the key role of vasopressin[49] in maintaining somatic survival for more than a few days following brain death. The importance of changing the focus of management from that of minimising the effects of cerebral trauma prior to the patient becoming a donor to that of optimal cardiovascular support, has been recognised elsewhere[110,111], but a crucial change in the regime described above has been the inclusion of a cardiac trained anaesthetist, who is skilled in the management of cardiovascular instability, as a member of the donor team, and the use of objective haemodynamic monitoring on which to base management and selection decisions has been central to the application of this strategy. The use of *continuous infusions* of HRT as opposed to bolus doses, has also been shown to be important. However, there are still a small number of donors (5% in this group) who are refractory to this management regime. The author has recently investigated the potential for physiologically resuscitating these donors using normothermic support bypass, with some success (Bibliography E)[112].

The finding that heart-lung recipients of HRT managed donors leave hospital significantly earlier than those donors receiving standard management, is surprising. Whilst there are some differences in demographics, as shown in Table 3.9, the causes of death and the ischaemic times were comparable between the groups. However, the heart-lung group of patients is much more susceptible to minor changes in medical management, in general, and may consequently be demonstrating some subtle functional benefit from HRT, as yet not identified.

Given the interactive nature of pre-load, after-load and inotrope therapy on cardiac function, it is difficult to find a single comprehensive descriptor of donor heart function. However, in this approach, marginal donors have been differentiated on the basis of wide deviation from the general acceptance criteria despite being *classified* according to the first major condition by which they fail (Table 3.4). This study did not seek to delineate the effects of HRT from optimal routine management. However, there is considerable evidence from the pilot studies to attest to the efficacy of HRT (Chapter 2).

What is clear, however, is that an overall comprehensive approach, which combines the elements of meticulous intensive care with the specific interventions needed to manage the brain dead, is required if the optimum outcome is to be achieved. This also means that donors managed in this standardised, objective manner form an ideal population base for evaluating potentially improved preservation techniques, as is evidenced by the influence of HRT on providing uniformity in haemodynamic function (Table 3.6).

3.5 CONCLUSIONS

The most important factor to emerge from this work has been the necessity to employ comprehensive monitoring which provides objective data on which to base optimal donor management and objective assessment of cardiac function. This reduces the risks of retrieving unsatisfactory organs and excluding potentially satisfactory organs.

The specific interventions, outlined above, provide a mechanism for improving peri-operative cardiovascular function. This has a particular relevance to the quality of

retrieved donor hearts, but has important implications for the viability of all transplantable organs. This study suggests that the numbers of satisfactory donor hearts may be increased by up to 30% and that the functional quality can be improved without compromising recipient outcome. It also means that this approach is essential if other potentially beneficial therapies are to be evaluated.

The following chapters explore a strategy for identifying potentially beneficial techniques in the more controlled environment of the laboratory. The more promising of these could then be subjected to clinical trial, using the regime described above in a standardised and managed clinical population.

CHAPTER 4

WORKING HEART MODELS

4.1 BACKGROUND

It has been broadly acknowledged that clinical studies of potentially improved therapies are difficult to evaluate in areas of medicine, such as transplantation. Numbers of patients are relatively small and the study environment very difficult to control. The author believes that the approach described in Chapter 3 is helpful in minimising these problems. However, before taking the step between theory and clinical trial, some form of pre-clinical testing is imperative. The following chapters detail one such possible strategy.

Methods for measuring myocardial functional integrity range from ultrastructural morphology[113], enzymology[114], biochemistry[56], histochemistry[39], and electrophysiology[97] to some measure of mechanical function[115]. Whilst the former may be useful in determining causes of specific malfunction, ultimately the heart is a pump which must be capable of performing sufficient work to maintain the normal circulation. Some reproducible measure of cardiac contractile function must therefore rank high on the list of desirable outcome measures in the assessment of cardiac preservation.

Cardiac contractile function can be assessed in many preparations within the laboratory, ranging from the whole heart within intact animals[47], to the single myocyte[116]. The isolated perfused heart model was first published by Langendorff in 1895[117] and has the distinction of having been in laboratory use, without interruption,

since that time. The basic model makes use of retrograde perfusion only, in either a constant-pressure or constant-flow modality. The most commonly used mode is that of constant pressure, since this is probably the best approximation to normal physiology[118]. Using a constant head of pressure in the aorta, and hence the coronary arteries, enables coronary flow to become one of the outcome measures[119].

Mechanical methods of sustaining isolated organs go back to Frey and Gruber[120] in 1885, who developed the principle of thin film blood-gas exchange, which continued to be developed by early twentieth century physiologists, culminating with Alexis Carrel and Charles Lindbergh's[121] pulsatile perfusion machine. Lindbergh had been stimulated to design a mechanical blood pump by his sister's, then untreatable, heart disease and he teamed up with the vascular surgeon Carrel at the Rockefeller Institute to produce a perfusion machine, eventually perfected in 1935. This device was capable of maintaining a functional guinea pig heart for 24 hours. This was the forerunner for the first clinical heart-lung machine developed by Gibbon and used successfully in 1952[122].

Further development in the perfusion of isolated organs, beyond the interests of the pharmacologists and physiologists, languished until around 1960 when the burgeoning interest in transplantation really began. Given that, at this time, the concept of brain death had not been accepted, it was recognised early on that the availability of a suitable organ perfusion system may enable many more organs to be salvaged. The deteriorating toxic milieu represented by a donor dying from circulatory arrest secondary to haemorrhage, respiratory arrest, poisoning etc. meant that it was difficult to obtain viable organs. Physiologically the main benefits to be gained from perfusing an organ are the supply of oxygen and nutrients, and the removal of carbon dioxide

and toxic metabolites. However, from a functional evaluation standpoint, some method of measuring work potential was required.

Contractile function is one of the most common end-points in studies of the effects of both pharmacological agents and pathophysiological processes in the heart. Three categories of cardiac functional assessment may be distinguished:

- ***Ventricular Performance*** which denotes the pumping process of the heart and may be measured in terms of cardiac output or work.
- ***Ventricular Function*** which relates ventricular performance to measures of preload, such as end-diastolic volume or end-diastolic pressure.
- ***Myocardial contractility*** which refers to a fundamental capability of the muscle to perform work.

A large number of different measurements have been used to provide approximate measures of contractility in intact hearts. Contractile force may be measured using an isotonic lever which is usually attached to the apex[123]. A better method for smaller hearts is to use a method which is not strictly isometric but rather auxitonic. The left ventricle is fixed rigidly to a strain gauge arch by two sutures and the right ventricle is connected to a force transducer. This arrangement allows the more circular distribution of the heart's force development to be registered[124]. Unfortunately, all show some degree of load dependence. The least load dependent techniques appear to be those derived from pressure-volume relationships[125], which give both qualitative and quantitative information on both systolic and diastolic function. However, these techniques are impractical in small mammalian hearts. Contractile function in small isolated hearts is most commonly assessed using an intra-ventricular balloon[126] but this method has a number of problems:-

1. The endocardium may be compromised by direct pressure.
2. The balloon may produce a false compliance.
3. The pattern of systolic shortening (against an effectively infinite afterload) is unphysiological.
4. The balloon may interfere with the mitral valve apparatus.
5. Balloons frequently produce rhythm disturbances.

However, this method is relatively simple and provides reproducible results[127]. Despite being in use for some 100 years, there is considerable diversity and conflict within the literature concerning the measurement of function in small mammalian hearts. These problems appear to be due to a combination of both methodological and semantic difficulties. In a recent survey of 50 publications from four leading Journals, published between 1986 and 1990, which used contractile function as a primary end-point, no two authors reported using identical methodology of preparation, perfusion or assessment[128]. This situation plays a role in the often conflicting results which arise from such publications.

Working heart preparations get around many of the limitations of the classical Langendorff model, described above. The first experiments using a *working* isolated perfused heart were described by Rigler in 1931 but most modern systems are based on that of Fallen et al[129]. In this preparation the Langendorff mode can be converted to the *working* mode by diverting perfusate into the left atrium and allowing ejection to occur via the aorta, in the usual fashion.

Working heart preparations, do get over many of the problems raised above but have a number of limitations of their own:-

1. It is difficult to separate afterload from coronary perfusion pressure as both are determined by the height of the afterload reservoir.
2. Flow through the left atrial cannula can limit cardiac output since filling only occurs in diastole; the LV diastolic pressure must be overcome, and fluid inertia may be a problem at high rates.
3. The aortic cannula has to offer minimal resistance if a stenotic obstruction is not to be produced.
4. Ventricular volume is not known and cannot be controlled, making it difficult to isolate systolic from diastolic functional changes. This is most pronounced following ischaemia[130]

Taking account of the above points, and using preload dependent *Function Curves* as the primary outcome measure, it is possible to have a reasonably sensitive model, with some measure of diastolic function. The technique does provide the overwhelming advantage of allowing large numbers of closely controlled experiments to be conducted quickly and relatively inexpensively, allowing control over the experimental procedure in the absence of the myriad of variables present in the clinical environment. In this way, the work reported in this chapter allowed a controlled study of key factors, the relevance of which will need to be determined in the clinical setting. The model described below was therefore developed as the primary discriminating measure for potentially improved preservation methods, in these experiments. Later on in these studies a large mammalian (and human) working heart system was developed as a potential pre-clinical model.

4.2 PERFUSION APPARATUS DESIGN CONSIDERATIONS

The apparatus was designed for testing adult guinea-pig hearts and is based on an earlier design which had demonstrated good stability and reproducibility[131]. A schematic of the system is illustrated below.

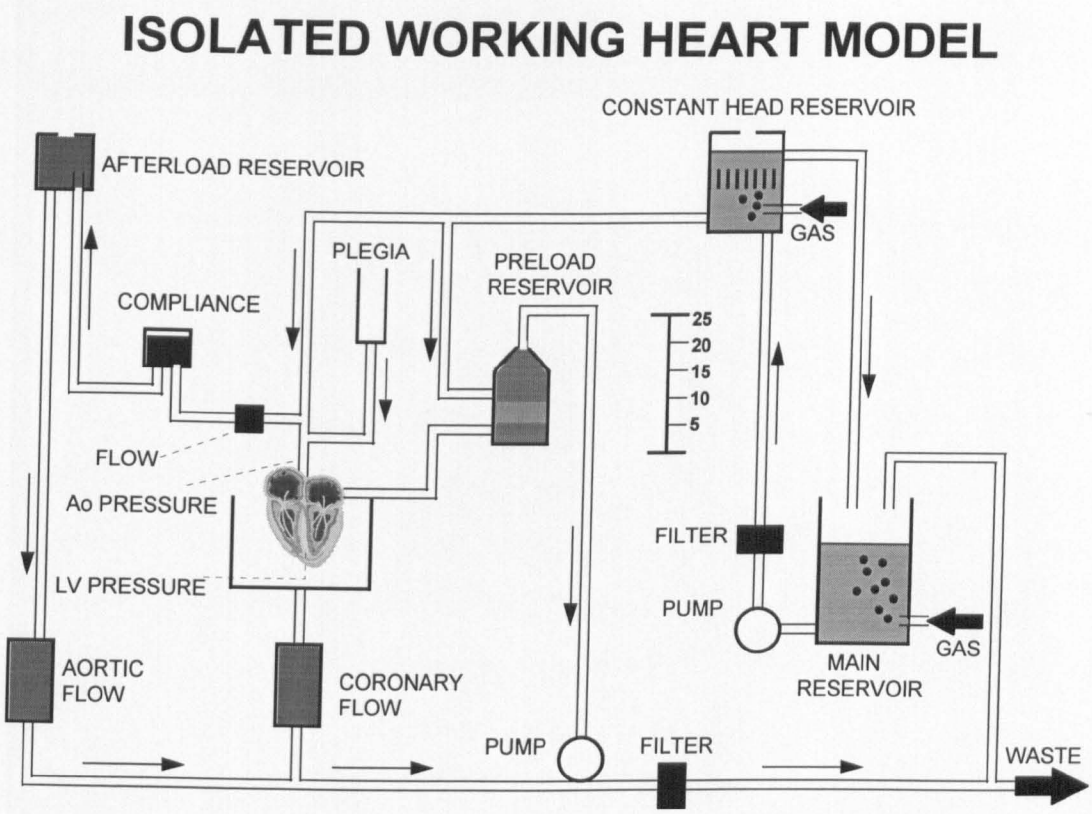


Figure 4A Schematic of the Guinea Pig working heart model developed for the screening of improved preservation methods. Perfusate is re-circulated, after an initial washout period and the preload is variable. Both antegrade and retrograde perfusion modalities are possible with this model. Cardiac function is measured by measuring aortic flow, with an electromagnetic flow meter, coronary flow by timed volume collection and the measurement of aortic and intra-ventricular pressures. Using these measurements, *Function Curves* can be constructed.

Essentially, the model consisted of a *Constant Head Reservoir*, which supplied both the aorta, during Langendorff perfusion, and the *Preload* (left atrial) *Reservoir*, during working mode. The height of the Preload Reservoir could be varied in relation to the left ventricle. Connection to the left atrium was via a 3 mm (ID) cannula which allowed a free flow of > 300 ml/min with a pressure head of 7.4 mm Hg (more than double the

maximum cardiac output). During the working mode, perfusate flows from the left atrium and is ejected into the working circuit via an electromagnetic flowmeter and a non-compliant 50 ml capacitance vessel placed 30 cm above the heart, which had a volume of 6.5 ml of air added. This was connected to an *Afterload Reservoir*, usually placed 70 cm. above the heart. Ejected fluid was collected from the *Afterload Reservoir* into a graduated chamber as was the coronary effluent.

Once the preparation was stabilised, the effluent perfusate was pumped back into a *Main Reservoir*, via a filter (Millipore SM 0.5 micron), where it was equilibrated with a 95/5% oxygen/carbon dioxide mixture, with further gas exchange taking place in the *Constant Head Reservoir*. To prevent cooling the heart was mounted within a water-jacketed chamber (38°C) and all the reservoirs contained heat exchangers supplied from the same temperature control system. The components were all connected by minimum lengths of 6 mm(ID) silicone rubber tubing. The system was primed with some 1.2 litres of perfusate. Since approximately 300 ml was directed to waste at the start of each set of function determinations, and a further 200 ml was flushed out following cardioplegic induction, about 50% of the perfusate was replaced with fresh solution for each experiment. Cardioplegia was administered via a small reservoir connected to the proximal aortic outflow, by means of a stopcocked side-arm. This reservoir was placed 50 cm above the aortic root.

Perfusate

The perfusate used for all of the following experiments, was a modified Kreb's-Henseleitt solution with composition as shown below:

TABLE 4.1

COMPONENT	g/l	mmol/l
NaCl	6.90	118.00
KCl	0.35	4.70
$CaCl_2$	0.28	2.52
$MgSO_4$	0.14	1.64
$NaHCO_3$	2.09	24.88
KH_2PO_4	0.16	1.18
D-GLUCOSE	1.09	5.55
Na-Pyruvate	0.22	2.00
EDTA	0.185	0.185

Sodium pyruvate was used as a substrate.
EDTA was added as a chelating agent for heavy metal contaminants.
The perfusate was used within 4 hours of having been made up.
All reagents were high grade Analar.

Cardioplegic Solutions

All cardioplegic solutions used in these and the following experiments, were made up by the NHS Regional Pharmacy in Ipswich. Some of these required the addition of insulin and/or oxygen, before administration. Cooled solutions were kept in the same monitored refrigerator pror to administration. Stored hearts were kept in a monitored cold room.

4.3 METHOD

The heart was instrumented with a Gould electromagnetic flowmeter, placed just distal to the aortic cannula. The aortic pressure signal was obtained from the distal aortic line, and the left ventricular pressure signal from a 20 swg needle placed in the lumen of the left ventricle. Both pressures were recorded via saline filled polyethylene

manometer lines to Gaeltec pressure transducers matched to a Roche Monitoring system. The frequency response of the system was well within the range required by the measurements (flat to < 20Hz). The heart rate was obtained from the aortic pressure signal via an instantaneous ratemeter. Electrical signals were recorded on a multi-channel hot pen recorder (Devices M19) and these were checked against manual records, at each measurement point. The aortic flow was checked against a timed graduated reservoir and the coronary flow measured in the same fashion.

Measurements

1. **Cardiac Output** was calculated from the sum of the Aortic and Coronary flows, meaned over a 1 minute interval.

2. **Stroke Volume** was calculated from the following relationship:

$$\frac{\text{Mean Cardiac Output}}{\text{Mean Heart Rate}} \quad \text{ml.min}^{-1}.\text{beat}^{-1}$$

3. **Left Ventricular Stroke Work** was calculated from:

(Stroke Volume x Mean Left Ventricular Pressure) x 0.0136 g.m.[130]

4. Initially **dP/dt max** was used but this was later abandoned in favour of the simpler and more sensitive measurement of Stroke Work[132]. The intra-ventricular pressure measurement was used in order to record mean left ventricular and end-diastolic pressures.

5. **Heart weight** was measured before and after drying in an 84^0C oven for >24 hours. All flow derived parameters were standardised to dry heart weight.

6 Control measurements were obtained for each animal for each experiment, in this series. Each heart therefore became it's own control.

Preparation

Male Dunkin-Hartley guinea-pigs (450-600g) were anaesthetised with intra-peritoneal Pentobarbitone. Approximately 25 minutes after administration, and following assessment of corneal reflexes, the animals were ventilated with air via a tracheostomy using a volume cycled ventilator. A sternotomy was performed and the major thoracic vessels isolated. The animal was heparinised (3mg/kg). The heart and lung block was removed under inflow occlusion (both cavae were ligated) and rapidly immersed in ice cold saline. Keeping the organs covered in cold saline, the pulmonary veins were ligated at their left atrial junctures, the pulmonary artery transected at the bifurcation and the lungs removed. The aorta was cannulated and the heart mounted on the working heart apparatus. The process of excision to mounting on the apparatus was typically accomplished in under 5 minutes.

Procedure

Having mounted the heart on the apparatus, retrograde flow from the header reservoir was allowed to flow under reduced pressure (29.6 mmHg) for about two minutes, before allowing full pressure to be applied. It was usual for spontaneous defibrillation to occur within 3 minutes of re-perfusion. During this period the coronary effluent was discarded to waste.

The heart was allowed to beat, under retrograde perfusion for 5 minutes, during which time the left atrial appendage was cannulated. To switch from retrograde Langendorff mode to working mode, the preload reservoir was adjusted to +3.7 mmHg, and the afterload limb of the circuit opened whilst still keeping the retrograde circuit open. After a few minutes the retrograde limb of the circuit was clamped and the preparation fully converted to the working mode. Following stabilisation for a further 5 minutes, function

curves were generated by incrementally adjusting the preload reservoir between 0 and 18.5 mmHg in increments of 3.7 mmHg . Values were recorded when stable, usually within 2 minutes of each change. At the end of a function curve run, the preload was returned to the 3.7 mmHg position. The average duration of a function curve measurement sequence was approximately 20 minutes. If cardioplegic arrest was required, this was administered via the *Cardioplegia Reservoir* in the following sequence:

1. The heart was returned to non-working mode by clamping the LA line.
2. The cardioplegia stopcock was opened while simultaneously clamping the distal aortic line.
3. The coronary effluent was directed to waste.

4.4 PILOT STUDIES

A series of pilot studies were carried out to prove the method in terms of default settings, reproducibility and stability (Figures 4B to 4E). Six guinea pig hearts were studied with respect to; heart rate, cardiac output, stroke volume and stroke work, over a sixty minute period in order to assess the stability and reproducibility of the model.

PILOT STUDIES
HEART RATE

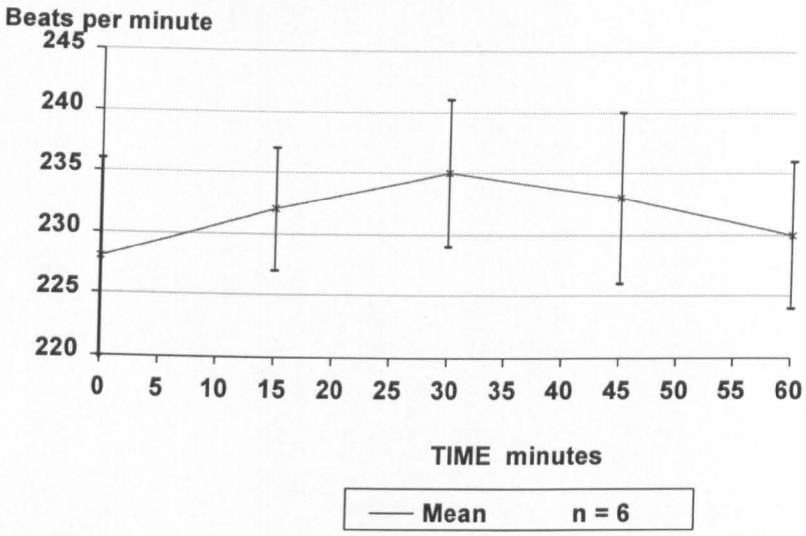


Figure 4B Heart rate remained relatively stable over the one hour evaluation period. Mean values for six hearts are shown above.

PILOT STUDIES
CARDIAC OUTPUT

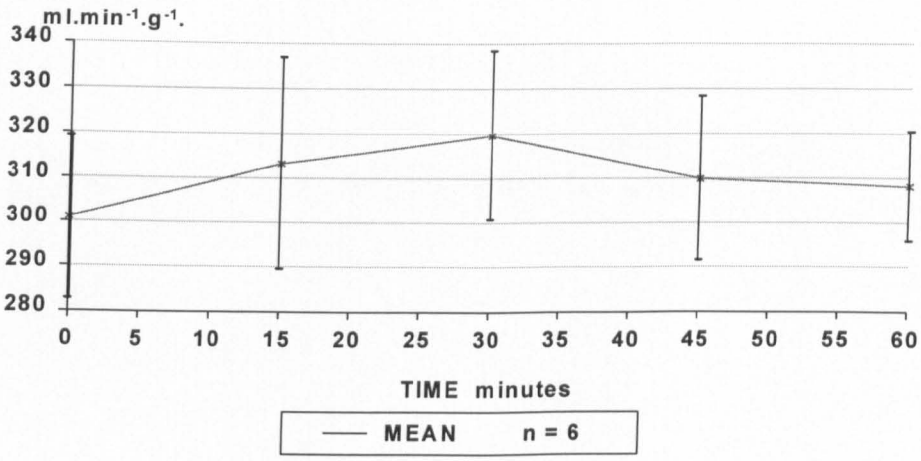


Figure 4C Six hearts were studied over a period of 60 minutes. Function studies were conducted every 15 minutes (Figs 4D and 4E), at which time the maximum heart rate and cardiac output was recorded. Mean cardiac output values are shown above.

PILOT STUDIES
FUNCTION CURVES

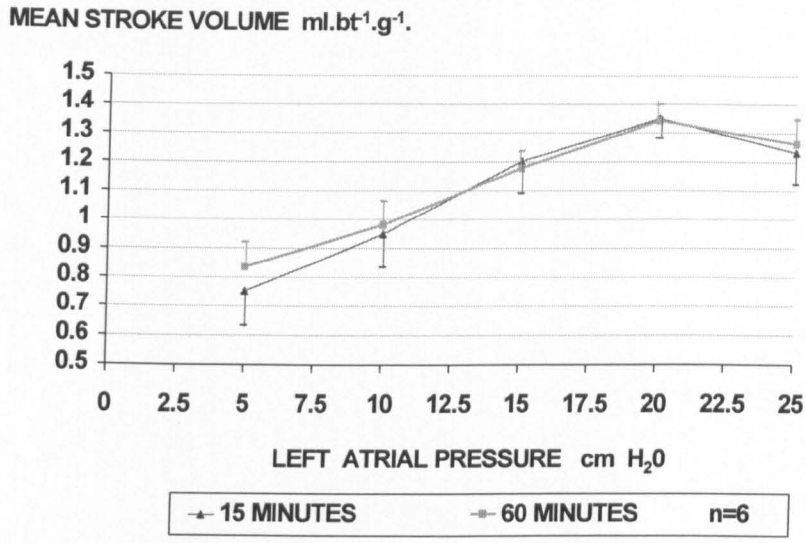
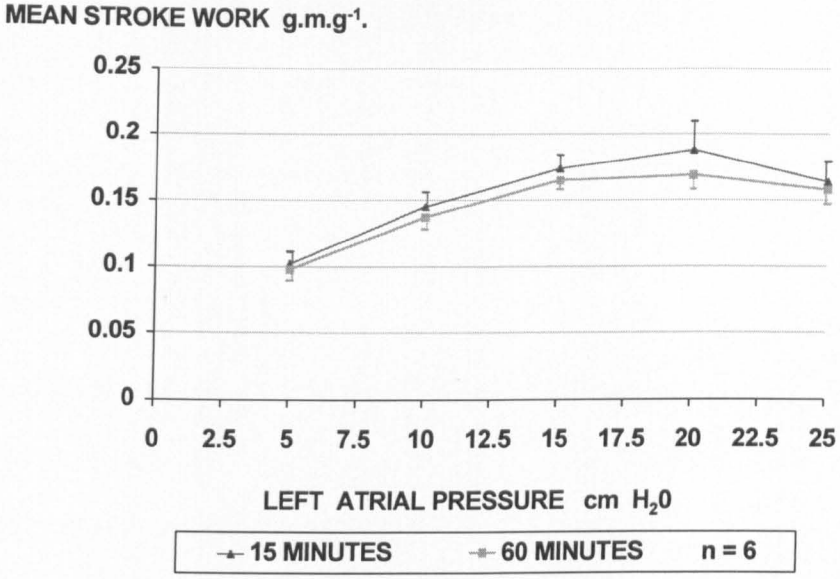


Figure 4D Stroke volume changes in response to incremental changes in left atrial pressure were measured at 15 minute intervals, up to 1 hour, in six guinea pig hearts. In between function curves the hearts were left in working mode at a left atrial pressure of 3.7 mmHg (1 cm H_2O preload = 0.74 mmHg). Only the 15 minute and 60 minute curves are shown, for clarity.

FUNCTION CURVE DATA TABLES
MEAN STROKE VOLUME VS LEFT ATRIAL PRESSURE WITH TIME

	PERFUSION TIME Minutes				
LAP mmHg (cm H_2O)	0	15	30	45	60
3.7 (5.0)	0.701	0.752	0.825	0.843	0.837
SEM	(0.112)	(0.118)	(0.093)	(0.071)	(0.085)
7.4 (10.0)	0.925	0.949	0.966	0.977	0.981
SEM	(0.103)	(0.112)	(0.093)	(0.072)	(0.063)
11.1 (15.0)	1.097	1.201	1.189	1.197	1.178
SEM	(0.108)	(0.110)	(0.082)	(0.077)	(0.059)
14.8 (20.0)	1.300	1.350	1.365	1.355	1.342
SEM	(0.101)	(0.067)	(0.077)	(0.062)	(0.059)
18.5 (25.0)	1.221	1.232	1.257	1.249	1.263
SEM	(0.119)	(0.110)	(0.091)	(0.069)	(0.085)

PILOT STUDIES
FUNCTION CURVES



STABILITY WITH TIME

Figure 4E Stroke work changes in response to incremental changes in left atrial pressure were measured at 15 minute intervals, up to 1 hour, in six guinea pig hearts. In between function curves the hearts were left in working mode at a left atrial pressure of 3.7 mmHg (1 cm H_2O preload = 0.74 mmHg). Only the 15 minute and 60 minute curves have been shown, for clarity.

MEAN STROKE WORK VS LEFT ATRIAL PRESSURE WITH TIME

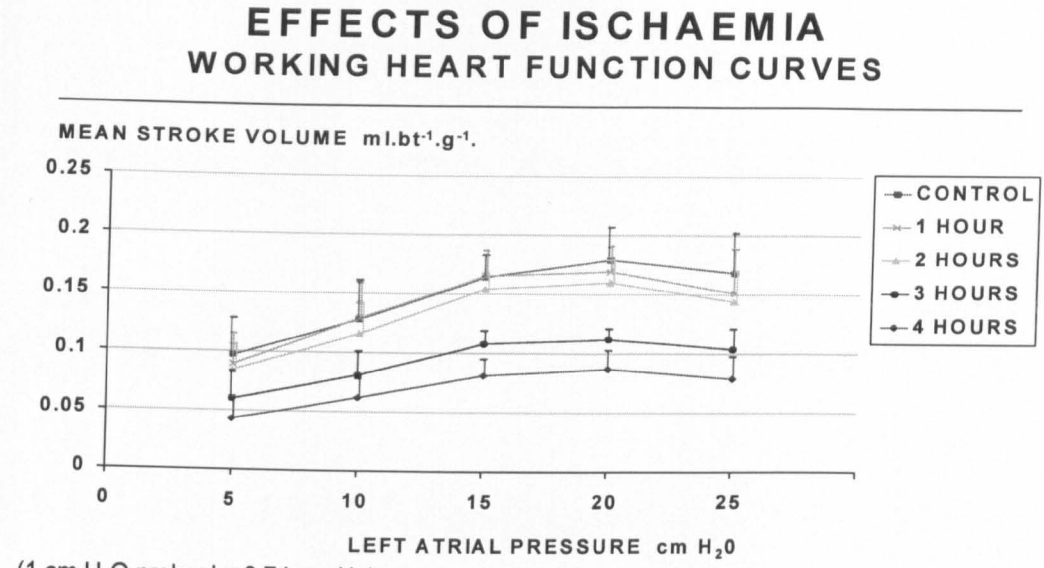
	PERFUSION TIME Minutes				
LAP mmHg (cm H_2O)	0	15	30	45	60
3.7 (5.0)	0.097	0.104	0.099	0.098	0.094
SEM	(0.031)	(0.028)	(0.029)	(0.029)	(0.032)
7.4 (10.0)	0.128	0.139	0.136	0.134	0.135
SEM	(0.033)	(0.030)	(0.031)	(0.024)	(0.027)
11.1 (15.0)	0.164	0.179	0.181	0.179	0.176
SEM	(0.029)	(0.032)	(0.031)	(0.027)	(0.024)
14.8 (20.0)	0.179	0.184	0.188	0.186	0.189
SEM	(0.038)	(0.037)	(0.039)	(0.026)	(0.025)
18.5 (25.0)	0.168	0.164	0.169	0.174	0.171
SEM	(0.032)	(0.037)	(0.029)	(0.028)	(0.032)

4.5 VALIDATION

Having evaluated the model with respect to reproducibility it was necessary to determine a storage time which would produce significant post storage depression of function without rendering the model unreliable. Twenty four hearts were divided into 4 groups of six. Control function curves were obtained, as described above, following which the hearts were arrested with St Thomas' cardioplegia (15 ml/kg body weight) at 4°C, and stored for periods of 1 to 4 hours in saline at 4°C.

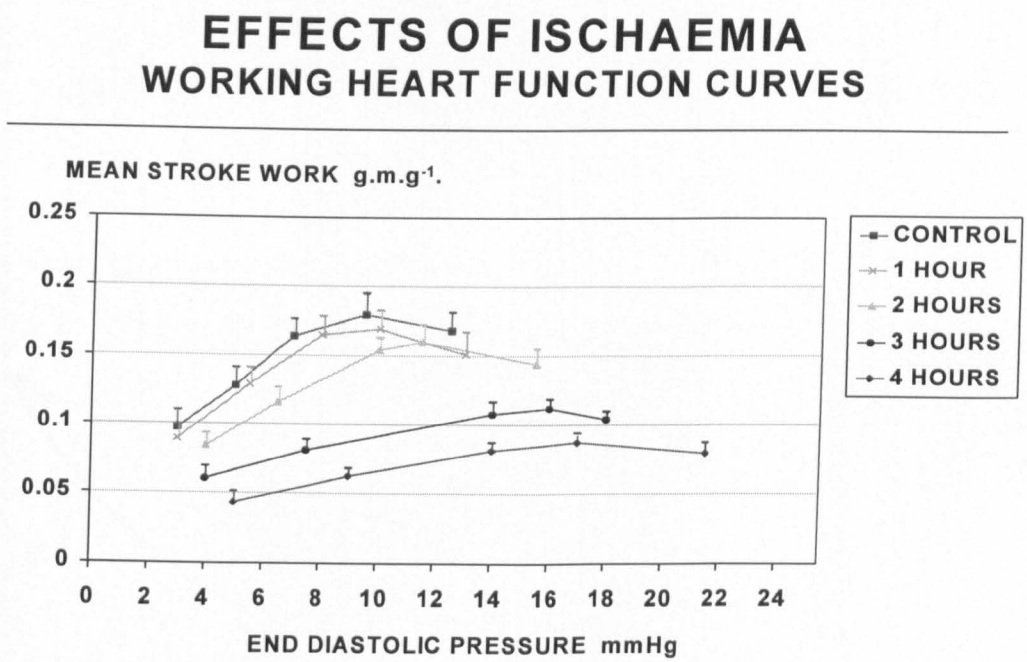
Following storage the hearts were remounted on the apparatus and function curves generated as before. On completion, the hearts were removed from the aortic cannula, blotted dry, weighed and placed in an 84°C oven for > 24 hours.

The function curves illustrated below (Figures 4F and 4G) demonstrate the deleterious changes in function which occur with increasing ischaemic times, using one of the standard cardioplegic solutions for myocardial protection.



(1 cm H_2O preload = 0.74 mmHg).

Figure 4F Twenty four guinea pig hearts were mounted on the working heart model and control function curves obtained for stroke volume at incremental changes in left atrial pressure. The hearts were arrested with St. Thomas' and stored at 4°C in saline. The hearts were randomly assigned to 4 groups for storage for 1-4 hours, after which post storage function curves were obtained. Values obtained at 3 and 4 hours were statistically significantly different from the control values ($p< 0.01$).



(1 cm H_2O preload = 0.74 mmHg).

Figure 4G This figure utilises the same data as that for Figure 4F but uses the end diastolic pressure generated at each LAP increment, to illustrate the change in diastolic compliance.

DATA TABLES RELATING TO THE WORKING HEART FUNCTION CURVE FIGURES

MEAN STROKE WORK VS LEFT ATRIAL PRESSURE WITH STORAGE TIME

	STORAGE TIME HOURS				
LAP mmHg (cm H_2O)	0	1	2	3	4
3.7 (5.0)	0.097	0.089	0.084	0.060	0.043
SEM	(0.013)	(0.011)	(0.009)	(0.009)	(0.008)
7.4 (10.0)	0.128	0.129	0.116	0.081	0.062
SEM	(0.013)	(0.012)	(0.011)	(0.008)	(0.006)
11.1 (15.0)	0.164	0.165	0.154	0.108	0.081
SEM	(0.012)	(0.013)	(0.009)	(0.009)	(0.006)
14.8 (20.0)	0.179	0.169	0.160	0.112	0.087
SEM	(0.016)	(0.013)	(0.012)	(0.007)	(0.007)
18.5 (25.0)	0.168	0.151	0.144	0.104	0.079
SEM	(0.013)	(0.016)	(0.011)	(0.006)	(0.008)

MEAN END DIASTOLIC PRESSURE VS LEFT ATRIAL PRESSURE WITH STORAGE TIME

	STORAGE TIME HOURS				
LAP mmHg (cm H_2O)	0	1	2	3	4
3.7 (5.0)	2.93	3.10	3.81	3.93	4.88
SEM	(0.00)	(0.10)	(0.80)	(0.70)	(1.60)
7.4 (10.0)	5.15	5.25	6.31	7.44	8.98
SEM	(0.70)	(0.80)	(1.20)	(1.10)	(2.10)
11.1 (15.0)	7.23	7.92	9.81	13.80	14.21
SEM	(1.20)	(1.10)	(1.20)	(1.80)	(2.20)
14.8 (20.0)	9.65	10.24	11.65	15.93	16.92
SEM	(1.40)	(1.60)	(2.30)	(1.20)	(3.10)
18.5 (25.0)	12.4	13.20	15.72	17.93	21.61
SEM	(2.80)	(2.10)	(3.10)	(3.90)	(4.30)

For statistical purposes the outcome measures of Cardiac Output, Stroke Volume and Stroke Work can be expressed as maxima or in terms of means across the preload range, as a measure of systolic function. As an indication of diastolic function the preload at which the maxima is achieved (LAP_{max}) can be used, or the left ventricular end-diastolic pressure can be plotted against the systolic parameter (Figure 4G). For most studies the pre-storage value has been used as the covariate.

4.6 HUMAN WORKING HEART MODEL

Whilst the model described above provides a valuable inexpensive and fast method for screening potentially improved preservation methodologies, it suffers from a number of deficiencies when relating to the clinical situation. These revolve around species differences and the use of a non-haem perfusate. The author therefore considered it prudent to develop a human working heart model to use for pre-clinical screening of the most promising techniques, identified using the small animal working heart.

With this in mind, the author initially developed a derivative of the Westerhof three element Windkessel model[133] attracted by the ability to tune the afterload to near physiological characteristics[134]. However, after a few attempts to obtain measurable work from explanted human hearts mounted on this apparatus, it was abandoned because of the technical difficulties associated with supplying sufficient blood flow to the left atrium. The simplified system, described below, was developed as an alternative.

Method

The system illustrated in Figure 4H was developed from an earlier device designed as a normothermic perfusion preservation system[135]. The circuit comprised a 4 litre polycarbonate chamber with a flexible silicone rubber lid through which the vascular connections to the heart were made. Effluent perfusate flowed to a flexible reservoir from where it was pumped by a paediatric centrifugal pump back to the aorta via an infant membrane oxygenator, heat exchanger and 40 micron filter. There was a shunt line which allows the perfusate to be circulated without the heart in circuit. Connectors in the arterial line allowed for pressure, temperature and perfusate flow monitoring and for samples to be aspirated. Additional monitoring was provided for monitoring carbogen flow to the oxygenator and temperature control of the waterbath, which supplied the heat exchanger.

HUMAN WORKING HEART MODEL

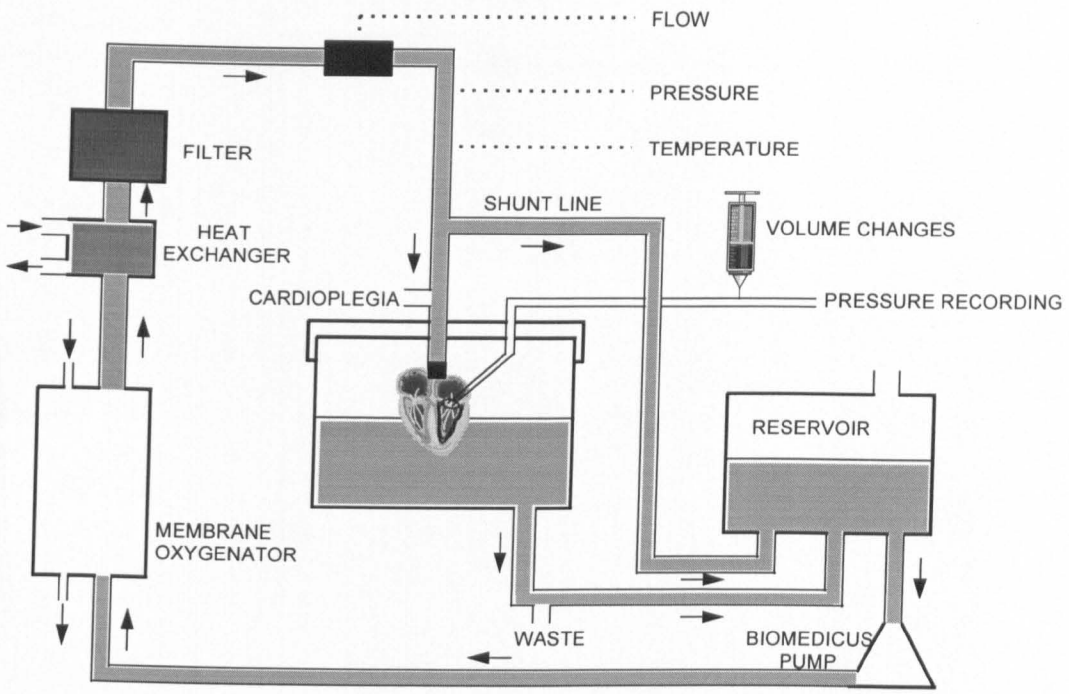


Figure 4H The above schematic illustrates the large (human) working heart apparatus constructed from paediatric perfusion equipment. The system is capable of supporting functioning hearts for more than 16 hours.

A series of pilot studies with sheep and pig hearts were carried out in order to establish a suitable perfusate and operating protocol. The composition of the final perfusate is given in Table 4.2. Blood was obtained by exsanguination from the animals and from the cardiopulmonary bypass machine, with additional crossmatched concentrated red cells, for the human experiments. All blood products were washed in a cell washer before reconstitution with the remainder of the perfusate, and then divided into two equal volumes. One half was used for the control study while the second was refrigerated pending the post storage studies.

TABLE 4.2

PERFUSATE COMPOSITION

COMPONENT	VOLUME ml
Washed Autologous Red Cells	800
Ringers' Solution	1000
Mannitol 20%	40
Glucose 50%	40
Sodium Bicarbonate 8.4%	30
Insulin	0.4 IU

The perfusate was constituted to the above formula before initiating perfusion. Half of the solution was used for the control studies and the other half refrigerated for the post storage studies. Once the circuit had been primed and brought up to temperature, oxygenator gas flow was adjusted, potassium, calcium and sodium bicarbonate added, if required, to establish normal values. An insulin infusion of 1 IU/hr was maintained throughout the course of the perfusion.

Hearts were arrested in situ with St Thomas cardioplegia and then transferred to the perfusion machine in cold saline. While the circuit was being primed and brought up to temperature a balloon was mounted onto a delrin ring which was secured in the mitral valve annulus by means of a purse string suture. Access to the balloon lumen was by a central luer port, connected to a manometer line and hence pressure monitoring equipment. Pacing wires were inserted in the left ventricle. A disc shaped aortic cannula was secured in the aortic root and the heart suspended from the flexible lid by means of this cannula and atrial sutures.

The heart was then connected to the circuit and reperfusion commenced with an aortic pressure of 20 mmHg and temperature of 35°C for the first minute. The perfusion pressure was subsequently increased in 10 mmHg increments each minute up to 50

mmHg. The perfusion pressure was then stabilised at 50 mmHg for five minutes, the temperature increased to 37°C and ventricular pacing commenced at 60 bt/min. The pressure was increased to 60 mmHg for a further 5 minutes and the heart defibrillated if spontaneous defibrillation had not occurred.

The pressure was next increased to 70 mmHg, pacing rate to 90 bt/min and maintained at these values for the duration of these subsequent experiments. Following optimisation of biochemistry and blood gas values, control function curves were obtained.

Functional evaluation involved the generation of pressure/volume curves by measuring systolic and diastolic pressure changes in response to incremental balloon volume changes. Volume increments were continued until there was no further increase in developed pressure. The balloon volume was subsequently returned to zero and the heart arrested with the technique under investigation.

During the storage period the perfusion system was emptied and flushed with Ringers solution. If the storage period was more than about two hours the system would be sterilised with Renalin (an oxidising solution used for renal dialysis filter sterilisation).

Following storage, the heart was connected to the circuit in exactly the same manner, as described above, except that an initial flush volume of approximately 200 ml was discarded during the first phase of reperfusion.

Post storage functional studies were conducted as per the control studies.

Using the above method it was possible to obtain measurable function following up to 12 hours preservation of the diseased human hearts and 16 hours in the animal hearts.

Results

Overall 32 large hearts were studied. These comprised 15 sheep, 12 pig and 5 human hearts. These pilot studies were conducted with a view to establishing the model, using four different types of preservation solution, in order to explore the limits of the technique. Having established the model a series of experiments, comparing a variety of preservation techniques were set up.

Figures 4I-4K illustrate some representative results from each species in these studies. In the controls the maximum balloon volume was limited to that which produced an End Diastolic pressure of 25 mmHg, so as to avoid over distending the ventricle, prior to storage.

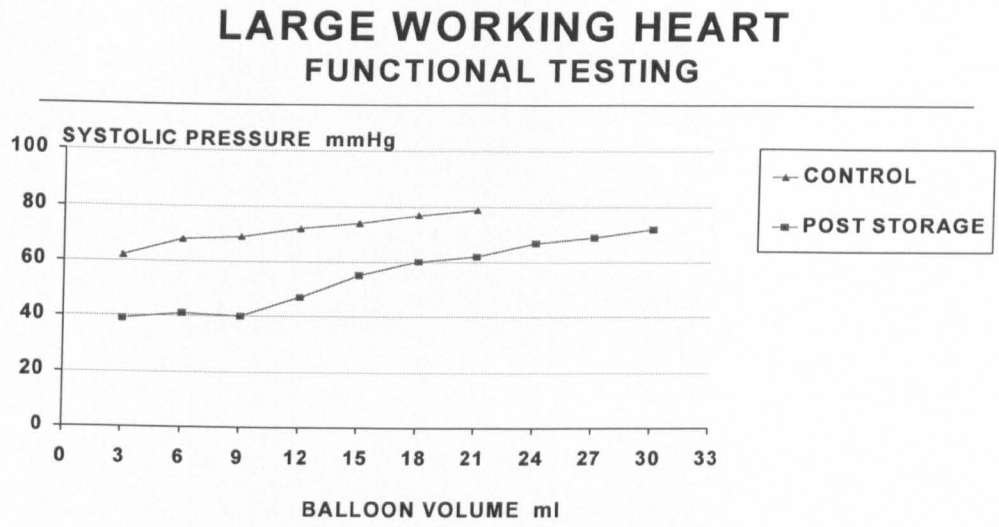


Figure 4Ia

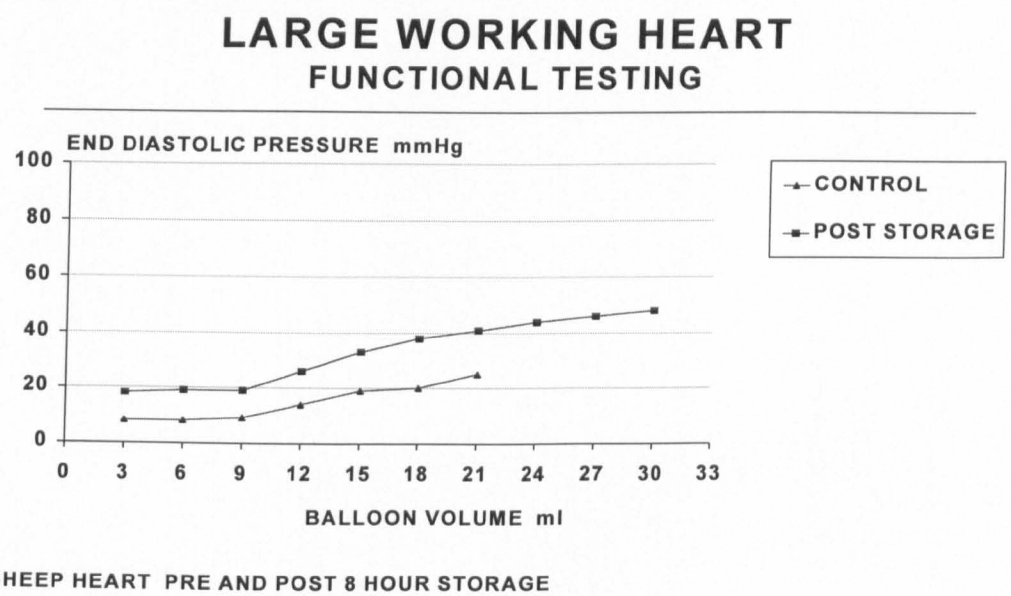


Figure 4Ib

Figures 4Ia and 4Ib These figures shows the incremental balloon pressures, in systole (Figure 4Ia) and diastole (Figure 4Ib), in response to incrementally volume loading the intraventricular balloon. Studies were conducted prior to and following 8 hour storage of a sheep heart in *Plegisol, in this instance. Both systolic and diastolic function can be seen to be depressed.

*See Chapter 5

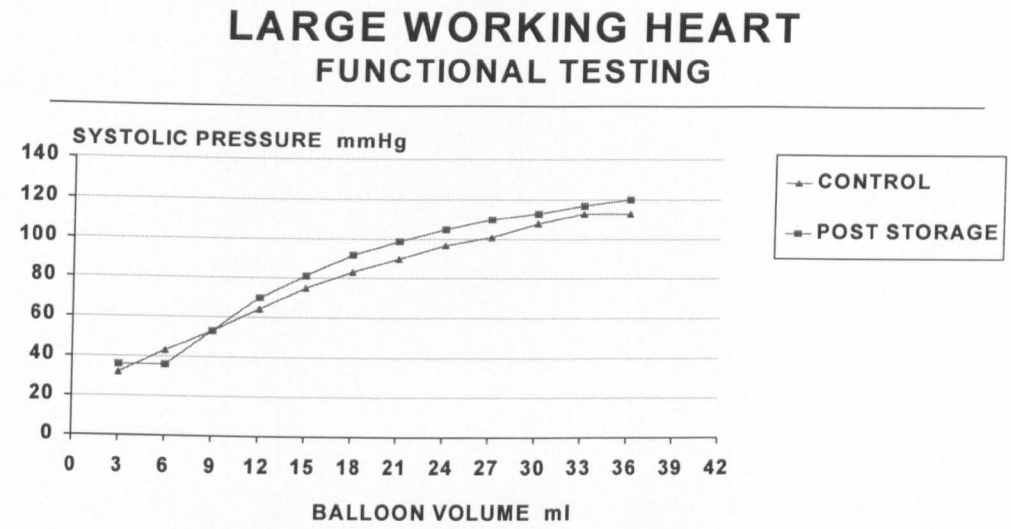


Figure 4Ja

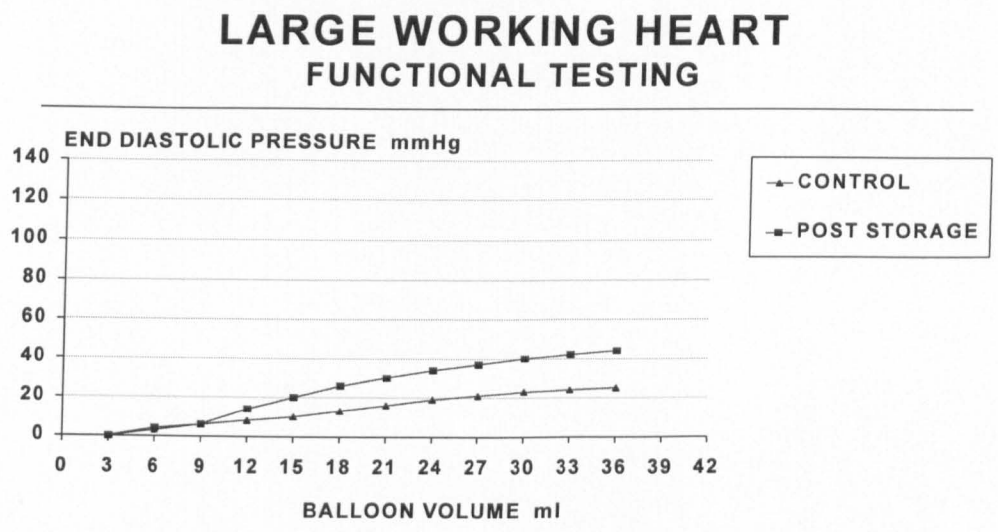
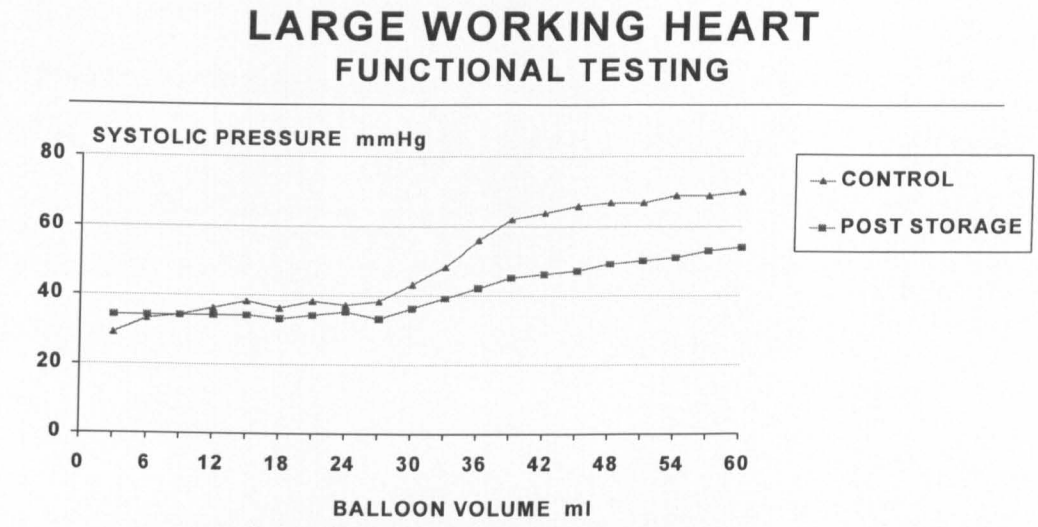


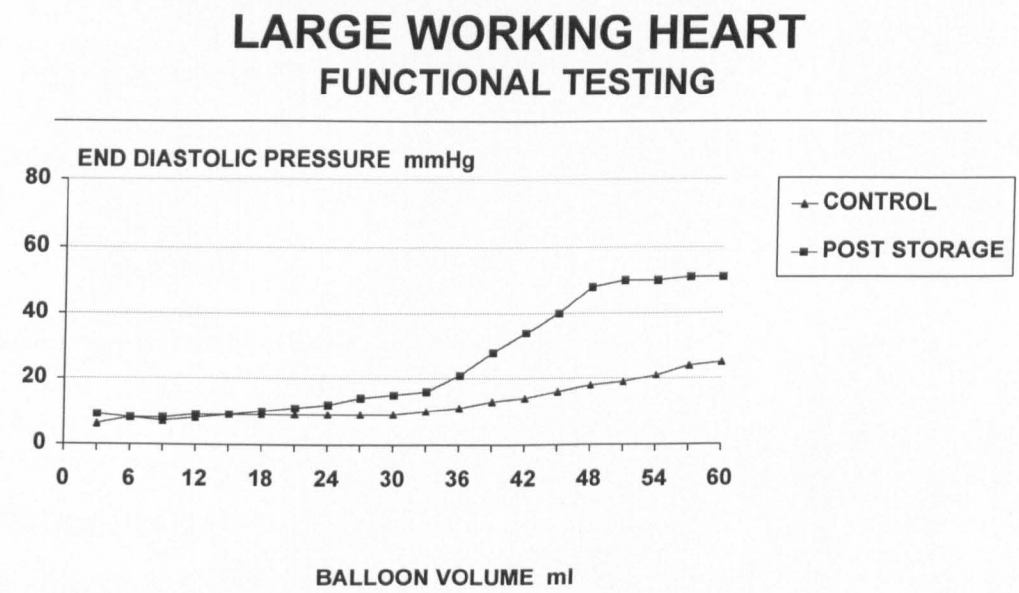
Figure 4Jb

Figures 4Ja and 4Jb These figures show comparative results with a pig heart subjected to identical experimental conditions as that shown in Figures 4Ia and 4Ib. Overall functional preservation was much better sustained in this species.



HUMAN HEART PRE AND POST 5 HOUR STORAGE

Figure 4Ka



HUMAN HEART PRE AND POST 5 HOUR STORAGE

Figure 4Kb

Figures 4Ka and 4Kb

These figure shows the equivalent results with a human heart, explanted because of end stage dilated cardiomyopathy. Despite advanced disease it was possible to obtain reasonable work from this preparation.

For comparative purposes systolic function can be compared with respect to differences in the systolic pressure at a given preload or the developed pressure (systolic - diastolic) at a given preload. Diastolic function can be compared with respect to compliance differences (balloon volume/EDP). An illustration of this is given in Table 4.3. Values are for balloon volumes of 20 ml in the sheep, 30 ml in pigs and 60 ml in humans.

TABLE 4.3

FUNCTIONAL EVALUATION

PARAMETER	DIFFERENCE BETWEEN PRE - POST STORAGE VALUE		
	SHEEP	PIG	HUMAN
SYSTOLIC PRESSURE mmHg	15	5	16
DEVELOPED PRESSURE mmHg	33	7	42
COMPLIANCE ml/mmHg	0.33	0.75	1.18

4.7 DISCUSSION

There are a number of laboratory based methods of evaluating myocardial viability and function, some of which could have a bearing on donor heart preservation.

Organ bath studies on various heart fragments, such as papillary muscle, ventricular or atrial strips, or septal preparations can provide the facility for looking at large numbers and provide a means of investigating human tissue. In the case of the latter there is a

limitation in that the material will invariably come from diseased human hearts and in the former, there is little advantage over the intact whole heart. A general criticism is that the tissue is *perifused* rather than *perfused*[136].

A number of studies into the mechanisms of anoxia and ischaemia have been undertaken using cultured preparations of either foetal hearts or cell monolayers. The use is somewhat limited with respect to surgical studies; foetal hearts differ from adults with respect to both electrophysiology and energy metabolism[137]. Cell monolayers are however useful for studies of toxicity[138].

Studies of isolated intact cells and subcellular organelles have, for many years, formed the basis for basic biochemical investigation into cellular and organ function. These preparations can be used for the study of ion flux and enzymology, but oxygen deprivation can only be studied by anoxia, rather than ischaemia. However, it is also worth bearing in mind that the interpretation of these studies has to take into account the interrelationships between various organelles, not represented by these models[138].

Any process of tissue damage or impairment of myocardial function must ultimately have it's origins in cellular chemical changes. Hence, biochemical markers can both identify the nature of an injury and potentially give an index of the degree of damage. This may take the form of unusual accumulations of ions in the extracellular, cytoplasmic or intravascular space. The most useful of these are those materials involved in the energy metabolism of the cell. Of particular prominence amongst these, are the high energy phosphates. There is considerable evidence in several species and models that ATP is a major factor in, and possibly a determinant of, tissue injury[139].

However, the use of these substances as markers in the surgical setting is fraught with difficulties; compartmentalisation may give spurious results, the substances are very labile with rapid cellular turnover times, and low levels may be determined more by precursor availability rather than cellular integrity[140].

Myocardial enzyme leakage has long been used for the assessment of tissue injury, particular in relation to myocardial infarction. Cardiac specific isoenzymes such as creatine kinase MB have the potential for giving semi-quantitative information on the degree of tissue damage[141], but the assay is technically difficult and prone to error.

Morphological studies, principally using electronmicroscopy[142], give useful information on ultrastructural integrity, pointers to possible calcium metabolism anomalies or other reasons for dysfunction and some index of oedema. However, the method is prone to the introduction of artefact and only provides a *snapshot* of conditions at the time of biopsy. Cytochemical methods, such as quantitative birefringence[39] can be more useful in estimating in vitro contractile potential, but the method is only semi-quantitative and is operator dependent.

Changes in the electrocardiogram and the transmembrane action potential have been used to monitor ischaemic damage and recovery in the setting of clinical myocardial infarction[143]. However, the application of these techniques to surgically induced ischaemia is more difficult due to the complexity of the situation, the associated ionic disturbances and the presence of hypothermia.

A number of electronic techniques can be used to provide evidence of metabolic status and tissue integrity. These range from microelectrodes to measure tissue gas tensions

and pH[144], ion specific electrodes[145], mass spectroscopy[146] and nuclear magnetic resonance[147]. These latter techniques however, require large and expensive equipment, the resolution time of which may limit the measurement of dynamic processes.

As outlined above, a wide variety of models exist for the study of myocardial protection. These are *in vivo, ex vivo* or *in vitro* preparations, using tissue from a wide variety of species, including man. Differences clearly exist between models and species. Extrapolation from one to the other, and particularly to the clinical situation, requires caution. The selection of a particular model necessitates a compromise between suitability of the model and a host of practical considerations. It is the opinion of the author that whilst all of the above techniques may add complimentary information, particularly with respect to *mechanisms*, ultimately an intact working heart is required to screen cardiac preservation techniques.

The working small heart model described in this chapter performed with similar characteristics to other models reported in the literature, with respect to absolute values of functional parameters and to stability[131,148,149]. This model, however, has the additional feature of being designed to produce preload dependent function curves

The small animal working heart model, however, does suffer from a number of deficiencies in terms of representing the conditions pertaining to a clinical donor heart; the animals used were not brain dead and no attempt was made to simulate the physiological effects of this[50], as described in Chapter 3. The model employs functional measurement of the left ventricle only and assumes that the interventions under investigation will have equivalent effects on right sided function[94]. A blood based

perfusate is not used, thus eliminating any cell mediated reperfusion phenomena which might have an effect on subsequent function[150], and last but not least, the guinea pig may not adequately represent man in important aspects related to myocardial preservation.

The human working heart model was therefore developed as a method for pre-clinical screening for promising solutions and techniques identified with the small animal working heart. The primary intention was to have a model in which human hearts could be functionally evaluated. The only human hearts used in these preliminary pilot experiments were those diseased hearts explanted from transplant recipients. These hearts were obviously compromised by the underlying disease for which the patient was being transplanted, but conversely, had not been affected by the metabolic consequences of brain death. An additional constraint of the present model is the necessity of using a recognised clinical cardioplegic solution (St Thomas') for the initial in situ cardioplegic arrest.

The large animal hearts were used as a means of developing the model. Ultimately it is envisaged that the model might be used with donor hearts from multi-organ donors from whom hearts are refused for clinical use, on the grounds of age; currently over the age of 60 years. This would provide a method for evaluating techniques and solutions on human hearts from brain dead donors.

An interest in organ perfusion techniques was developed very early on by the scientific transplant community. In vivo normothermic perfusion, not surprisingly, provides ideal conditions for preserving organs and Angell and Shumway[151] demonstrated this

technique of an *intermediate host* in a dog model for 3 days in 1966. However, it is difficult to see how this may be translated into a clinically relevant technique.

In vitro perfusion is a more realistic approach, and beginning with Langendorff[117], has a history of over 90 years. However, despite an enormous experience with variants of this model there remain many unresolved problems, as is evident from the lack of widespread clinical application.

Normothermic perfusion is the most physiological, and by the same count, the most difficult technique to achieve for a clinically useful time. Using blood as a perfusate the system must be closed in order to keep infection at bay, the supply of substrate and the removal of toxic metabolites need to be addressed. Normothermic perfusion is, however, imperative for functional testing.

A variety of attempts have been made to effect a compromise in this situation; Robicsek[152](1968) has used a simple gravity system to maintain blood perfused hearts for up to 12 hours. Tam[153] extended this to 24 hours in 1971, by dialysing intermittently with cross circulation from another animal. Pitzele and Dobell[154] also reached 24 hours using a membrane oxygenator and frequent changes of blood. It is clear that whilst this may be a method for physiologically resuscitating and evaluating a heart, it is not a realistic method for preservation.

Perfusion *preservation* has been used experimentally for many years. Hypothermic (2 - 10°C) perfusion of the in vitro organ benefits from the reduction in metabolic rate at lower temperatures and enables a simplified perfusion system to be employed. Flow requirements are reduced, damage to the perfusate minimised and bacterial growth is

inhibited. The use of blood as a perfusate is fraught with problems at low temperatures and there is little benefit in doing so since crystalloid solutions are capable of delivering sufficient oxygen at these low temperatures.

In 1968 Belzer[155], after many problems with plasma as a hypothermic perfusate for the kidney, accidentally found that if the *cryoprecipitate* which resulted from thawing frozen plasma, was filtered out, the resultant perfusate was capable of maintaining kidneys viable for up to 3 days on a perfusion preservation machine. In 1969 Feemster and Lillehei[156], used the same technique, with the addition of hyperbaria to 3-4 atmospheres, to successfully preserve dog hearts for up to 24 hours. Subsequently the technique was used to preserve the dog pancreas for 24 hours and the liver for 48 hours[159]. Belzer's early success in perfusion preservation underpinned what might be called the *physiological* approach to organ preservation.

However, Proctor and Parker[158], called into question this physiological approach, arguing that for organs which have evolved to function at a very different and closely controlled temperature, *normal* physiology was irrelevant. Instead they adopted an empirical approach and developed a simple low-flow (pressure controlled) system employing a base Kreb's solution with added glucose and dextran. Copeland and Stinson[159] were later able to use this technique to obtain long term survivors after 48 hour preservation followed by orthotopic transplantation in dogs.

The technique was later adopted by Wicomb[160], in Cape Town using a much more complicated perfusate. After a series of successful 24 hour storage experiments in a baboon model, the system was used clinically in 1981, but with poor results. More

recently Wicomb[161] has turned his attention to a technique of micro perfusion, for extended organ preservation.

It is the opinion of the author that none of these techniques will find a place in the clinical arena unless a very simple and reliable system can be devised. This is exemplified by experience from renal transplantation where perfusion preservation systems have largely fallen into disuse, despite the fact that superior results have been obtained with these systems[162].

The message emerging from this would seem to be that normothermic in vitro perfusion provides the promise of providing the basis for a method of functional evaluation with the possibility of metabolic and physiological resuscitation. Perfusion preservation, on the other hand, is probably the most fruitful avenue to explore in an effort to obtain safe extended preservation times. However, for this to enter the clinical arena the device would have to provide simplicity and safety.

4.8 CONCLUSIONS

Having taken into account the various limitations of small working heart models, a system was devised and tested which seems to provide a simple yet effective mechanism for screening hearts subjected to global ischaemia. The model provides a stable preparation for at least 40 minutes, which is approximately twice the time required for the measurements described above. The ischaemic storage studies suggest that a storage time of 3 hours should provide adequate discrimination between methods.

Whilst this model has a number of limitations when compared to the clinical situation, the circumstances surrounding the conduct of multi-organ donor retrieval operations, as outlined in earlier chapters of this thesis, make it almost impossible to carry out well controlled targeted research. It is for this reason that the integrated approach adopted in these studies, is advanced as a mechanism for making progress in this difficult area. This small animal working heart model makes a significant contribution as a simple, fast and cost effective method of screening potentially useful new solutions and techniques[163]. Studies, using this model, are described in chapters 5, 6, and 7.

The feasibility of using a human working heart model for functional studies has been demonstrated. This could provide a pre-clinical validation model using hearts from multi-organ donors whose hearts are not deemed suitable for clinical transplantation, mainly on the grounds of age. This would provide hearts from brain dead individuals of the same species, thus removing many of the confounding elements present in current donor preservation models. In this scenario, it would be possible to obtain pre-excision functional data (Chapter 3 and Bibliography F) and to use the preservation technique under investigation. The author believes that the combination of this approach together with the improved donor management regime detailed in Chapter 3, provide the means for making a significant impact on what has been a most refractory problem, over the past 25 years.

CHAPTER 5

CARDIOPLEGIC INDUCTION

5.1 BACKGROUND

The normothermic human heart tolerates complete ischaemia for less than 30 minutes, after which profound functional and morphological injury becomes evident[164,165]. The early pioneers of cardiac surgery recognised that oxygen consumption declined as temperature fell[166] and this opened the way for the first generation of cardio-pulmonary bypass machines, which could not sustain normothermic oxygen delivery[167], but could manage the reduced flow and oxygen transfer required under hypothermia. The technique was taken to it's ultimate in the use of hypothermic circulatory arrest, to facilitate surgery for complex congenital defects in children[168].

Selective myocardial hypothermia has been shown to extend the period of safe anoxic arrest, in the setting of corrective cardiac surgery, by more than one hour, by several authors[165,169]. Several studies have demonstrated a two to four-fold decrease in myocardial oxygen consumption in the non-arrested heart, as the temperature falls from 37°C to 15°C[170,172]. However, since 80% of myocardial oxygen consumption arises from mechanical work[173], chemical arrest of the heart contributes to a very substantial further decrease in oxygen consumption, of the order of a further four-fold reduction[174,175], and the two are additive (Figure 5A). Once coronary blood flow has been interrupted, therefore, it is important that cardioplegic induction be achieved rapidly, with the minimum of energy, and hence substrate consumption, and without

physical or chemical injury. Following reperfusion, there should be a resumption of normal electromechanical function.

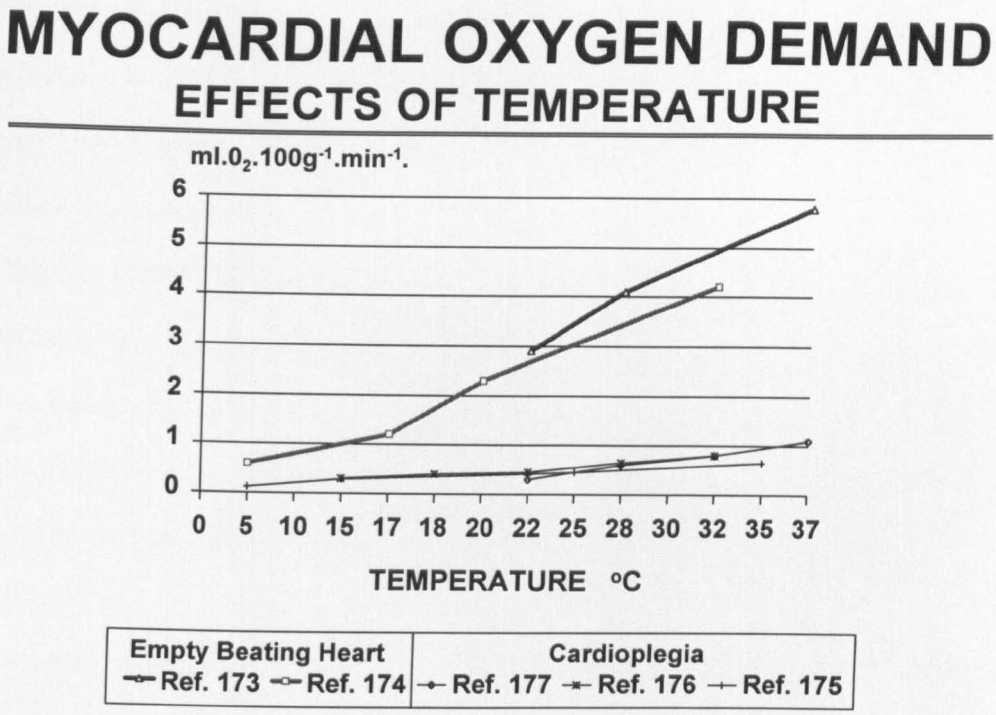


Figure 5A The curves illustrated above have been produced for data abstracted from the quoted references. Differences between the authors are mostly related to slight differences in experimental conditions but, overall it can be determined that the Q_{10} is approximately 2 and that the addition of chemical cardioplegia decreases the oxygen consumption by a factor of 6, at higher temperatures and by a factor of 2 at temperatures in the 4°C.range.

The concept of elective cardiac arrest was developed by Melrose and Bentall[176] in London in 1955. This was to overcome the problem during cardiac surgery, posed by a moving heart and an operative field obscured by coronary venous blood which was the consequence of keeping the coronary arteries perfused. After occluding the aorta they rapidly injected a 2.5% solution of potassium citrate in blood, into the aortic root to arrest the heart. They had previously shown, in a variety of animal models, that once

the aortic clamp was released potassium was washed out of the coronary system and the flaccid asystolic heart rapidly resumed normal electro-mechanical action.

The technique was taken up by numerous centres on both sides of the Atlantic[177-179]. At the end of the 60's, however, doubts began to be expressed about the safety of this procedure. A number of investigators reported associated experimental and clinical evidence of myocardial injury culminating in the findings reported from Bethesda[180] in which the hearts from 19 patients who had died following cardiac surgery showed marked myocardial necrosis in those who had received potassium citrate cardioplegia. The damage was subsequently shown to be due to the high potassium concentration in the Melrose solution (>250 mmols). The result was that cardioplegia effectively disappeared from the surgical literature for the subsequent 15 years.

The return of elective cardiac arrest, or cardioplegia as it had now been christened, was again the result of a European initiative. The concept had been kept alive in Germany mainly by Bretschneider[181] who published the principal of keeping the heart arrested with sodium-poor, calcium-free, procaine containing solutions. The rationale behind this formulation was that a sodium concentration equal to the intracellular sodium content would prevent the excitation potential, the lack of calcium ions would prevent activation of the contractile system, and that procaine would stabilise the cell membrane potential. Osmolarity was maintained with mannitol. Subsequently histidine was added as a buffer, because of it's superior buffering capacity at low temperatures.

Sondergaard[182] reported the first series of surgical cases using the Bretschneider approach, in 1975. A number of other Europeans adopted a similar approach as exemplified by Kirsch[183] who worked on the principal that an ideal solution should

primarily have Adenosine Triphospate (ATP) sparing characteristics and should therefore not contain calcium, potassium or sodium. Local anaesthetics and magnesium, being membrane stabilising agents, were believed to slow the decay of organic phosphate in the cell. The Kirsch magnesium-aspartate-procaine bolus solution came into regular use in several German centres during the early 70's.

Stimulated by this work, Hearse[184], working in London, tested the individual components of the Bretschneider and Kirsch solutions on an isolated working rat heart model. However, Hearse was unhappy with the unphysiological nature of these solutions and he advocated the principal that cardioplegic solutions should retain, as closely as possible, extracellular rather that intracellular concentrations of ions, with only those additions which could individually be shown to be effective, in their optimal concentrations. He proposed the St.Thomas' solution, based on a Ringers' base, to which was added 16 mmols potassium chloride, to effect rapid arrest, 16 mmols of magnesium chloride, which had, in this concentration been shown to have a marked additive effect and 1 mmol of procaine hydrochloride. This solution was introduced into clinical open heart surgery in 1975. More recently a variant of this solution, St. Thomas' 2, with half the calcium and a lower potassium concentration, was introduced under the commercial name of Plegisol[185].

Several workers in the USA began to report on a variety of potassium and magnesium based cardioplegic solutions[186,187] such that by the end of 1978, coronary perfusion, as a means of preserving the myocardium during surgery, had virtually disappeared from clinical practice. The discussion was then not whether, but what sort of cardioplegia should be used. There were three geographical groups in this respect; the continent of Europe, particularly east of the Rhine, used sodium-poor, calcium-free, magnesium

and procaine solutions, the UK and much of western Europe used the Ringer's based extracellular solutions and the USA used primarily potassium-enriched solutions containing little or no magnesium and no local anaesthetic.

With the advent of cardiac transplantation in the early 70's it was recognised that the heart was only tolerant to relatively short global ischaemic times. This meant that the early operations were all carried out with the donor and recipients in adjoining operating theatres[13]. However, in the late 70's the cardioplegic solutions being used for routine open heart surgery began to be used for preserving donor hearts, so as to avoid the logistical, ethical and emotional problems of transporting brain dead donors to the recipient hospital[188]. The fact that conditions prevailing during ischaemia for routine heart surgery and those during donor heart preservation and transport are very different, was ignored in the face of the security of adopting practices which seemed to work satisfactorily. With the rapid expansion of heart transplant centres there was a tendency not to challenge the existing techniques but merely to adopt the entire *package*. The group in Cape Town[46] was one of the few who attempted to develop transplant specific solutions and techniques, borne more out of necessity, due to their relative geographical isolation, than intellectual curiosity.

In this chapter, the effects of hypothermia alone are compared with hypothermia plus one of four different cardioplegic solutions, on the efficacy of cardioplegic induction for heart transplantation. The four solutions; Bretschneider HTP, St.Thomas' No. 1, Plegisol and Wicomb were chosen as representatives of the solutions discussed above. Wicomb solution represents the only solution developed experimentally for donor heart preservation and is essentially a glucose/insulin solution with Verapamil as a calcium antagonist[46].

The compositions of these solutions are given below in Table 5.1.

TABLE 5.1

CARDIOPLEGIC SOLUTIONS				
Solution	**Bretschneider HTP**	**Plegisol**	**Wicomb**	**St Thomas No.1.**
(mmol/l)				
Sodium Chloride	15	110	102	144
Potassium Chloride	8	16	10	20
Magnesium Chloride	8	16	-	16
Magnesium Sulphate	-	-	14	-
Calcium Chloride	-	1.2	1.1	2.2
Dextrose	-	-	278	-
Mannitol	20		-	-
Sodium Bicarbonate	-	10	-	-
Histidine	180	-	-	-
Histidine Hydrochloride	15	-	-	-
Tryptophan	2	-	-	-
Verapamil (µg)	-	-	1.5	-
Insulin (I U)	-	-	20	-
Osmolarity (mosm/kg)	287	300	385	350
pH at 4°C (pH units)	7.3	7.5	7.3	6.8

This Table gives comparative compositions of the four cardioplegic solutions investigated. Concentrations are expressed as milli mols per litre apart from Verapamil, which is expressed in micrograms and Insulin, which is expressed as International Units. Bretschneider and Wicomb base solutions were made up at the Regional Health Authority Central Pharmacy, in Ipswich. St. Thomas' No. 1 and Plegisol were obtained as commercial solutions from Macarthy Ltd, England.

5.2 METHOD

Heart-lung blocks were removed from 24 anaesthetised male Dunkin-Hartley (450-600 g) guinea-pigs, using the technique described in Chapter 4. Control function curves were obtained, prior to arrest, in order to confirm normal function. Cardioplegic induction was achieved with one of four solutions:

1. Bretschneider HTP
2. St.Thomas' 1
3. Plegisol
4. Wicomb

Cardioplegic induction was carried out in identical fashion for each heart. This consisted of:

- Returning the heart to Langendorf (non-working) mode.
- Clamping the distal aortic outflow.
- Administering 15ml/kg (body weight) cardioplegia, at a pressure of 40 mm Hg, at 4°C.

The groups were compared with respect to:

a. Time to arrest T_{ARR}

b. Coronary Resistance C_R

c. ^{31}P-NMR Spectroscopy

Coronary flow is not reported on, since this is reflected in the measurement of coronary resistance, with a fixed perfusion pressure.

These hearts were subsequently stored at 4°C for 3 hours, after which they were re-mounted on the working heart apparatus and post-storage function curves obtained, as described in Chapter 7.

Nuclear Magnetic Resonance (NMR) Spectroscopy provides a noninvasive method of following intracellular changes in high-energy phosphate levels and the accumulation of protons (decrease in pH). This means that functional studies can be performed on the *same* heart. In this study only limited access was available to the magnet, so limiting the number of experiments which could be conducted, precluding statistical analysis of results.

NMR Measurement method

The studies were performed using a Bruker AM-360 spectrometer with a vertical 8.5 T magnet. The radio frequency coil was tuned to 100.18 MHz for protons, to shim the static magnetic field and to the ^{31}P frequency of 40.65 MHz. Field homogeneity was adjusted using the water ^{1}H signal[189,190]. The 90-degree pulse length for ^{31}P was 80 μsec, and most spectra were accumulated with 50 μsec pulses applied at intervals of 1 second. Spectra were obtained by means of the Fourier transformation after 14 Hz line broadening. Gross baseline distortions were first corrected by manual subtraction of a quadratic polynomial fit. Simultaneously, Phosphocreatine (PCr) peaks were shifted to the same frequency for automatic processing comprising a baseline least-square linear fit. When PCr was not present water was effectively used as the reference frequency[189]. Peak area errors were in the 7% range. Values for pH were determined for the chemical shift difference between the inorganic phosphate and phosphocreatine signals, at 4°C with the following formula[190].

$$pH = 6.88 + \text{Log}_{10} \frac{\Delta - 3.35}{5.6\,\Delta}$$

Errors for the pH estimations were in the 0.15 pH unit range.

Eight additional hearts (two hearts from each group) were subjected to the same protocol as above and then analysed by NMR spectroscopy for intracellular energy metabolism and pH, measured at 20 minutes post arrest, having been stored at 4°C during this period. The NMR measurements were carried out by Dr. David Reid at Smith Kline Beecham.

A further series of 6 experiments were conducted to obtain qualitative information on the efficacy of hypothermia alone, as an inductive agent. For these experiments 4°C Kreb's solution was used as the perfusate.

Statistical Analysis

The outcome measures of T_{ARR}, and C_R were analysed by analysis of variance, taking into account the type of solution used. If a solution was found to have a statistically significant effect, then the solutions were compared pairwise, using Fisher's LSD method[191].

5.3 RESULTS

T_{ARR}

There was a statistically significant difference between the times to electromechanical arrest of the hearts induced with the four solutions ($p<0.001$).

The hearts arrested with Bretschneider HTP solution took approximately three times longer to arrest than those hearts induced with the other three solutions. Within these three remaining solutions, the hearts induced with St.Thomas' 1 took less time than the Plegisol (11 seconds) and Wicomb (7 seconds) less, respectively (Figure 5B).

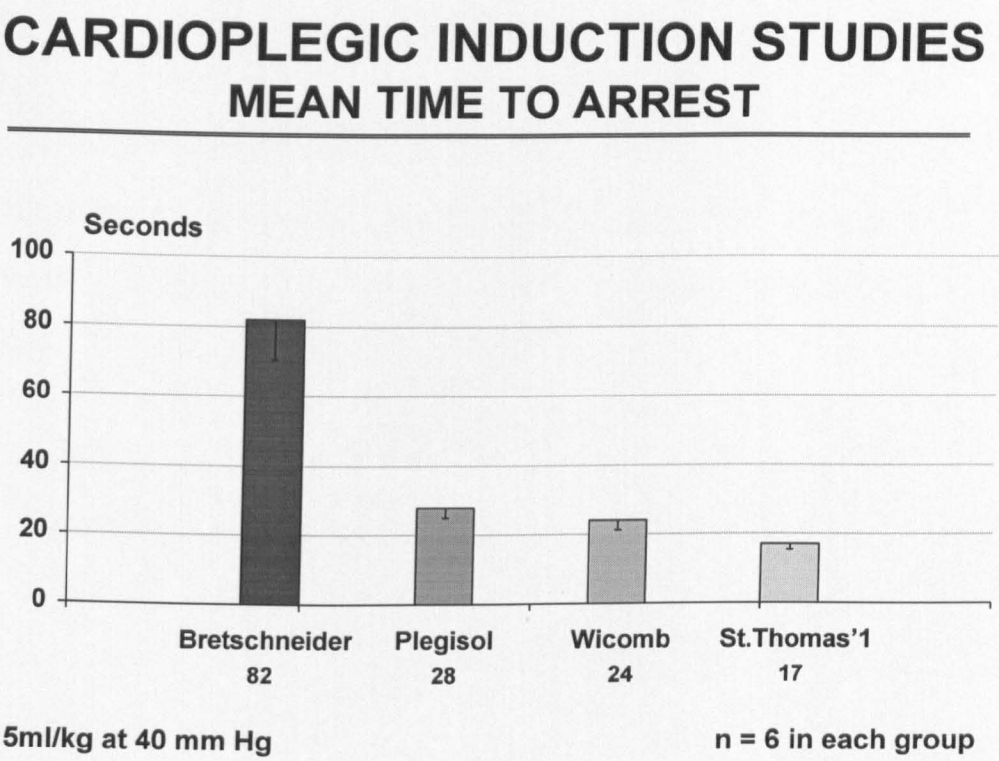


Figure 5B Histogram showing the differences in mean time to arrest with the four cardioplegic solutions, administered under identical conditions of volume, temperature and pressure. It is clear that Bretschneider HTP solution is much less effective than the other three solutions, as an inductive agent.

The mean time to arrest for the four solutions are ranked as follows:

St. Thomas' No.1 < *Wicomb* < *Plegisol* < *Bretschneider HTP*

In the above and in the following comparisons, solutions underlined by the same line are *NOT* statistically significantly different.

Figure 5C shows representative left ventricular pressure traces for each solution, which illustrates both the temporal differences in induction, produced by each, but also the nature of electromechanical work performed, during the anoxic period of induction. Bretschneider HTP solution showed gradual bradycardia with no loss of developed pressure and abrupt arrest. Plegisol and Wicomb solutions showed gradual bradycardia and an associated loss of developed pressure, before standstill. St. Thomas' 1, on the other hand, showed no bradycardia and an *increase* in developed pressure before an abrupt arrest.

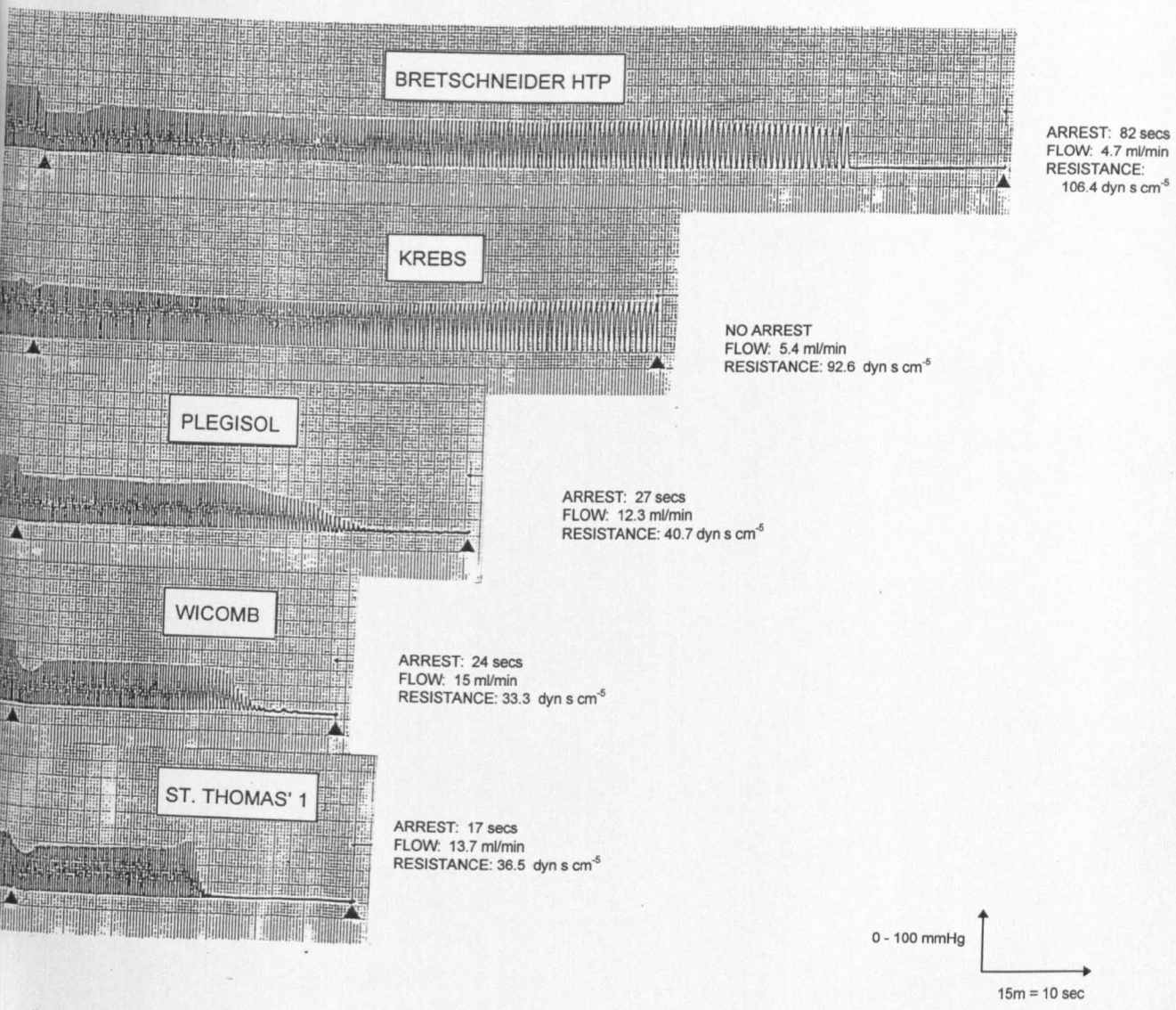


Figure 5C.
Cardioplegic Induction

Each solution was administered at 4°C and at a pressure of 40 mm Hg. The same volume - 7 ml - (representing 15 ml per kg body weight) - was administered during the period between arrows. Mean time to arrest, mean flow and mean coronary resistance is given next to each trace.

C_R

There was a statistically significant difference between coronary artery resistances between the induction groups ($p<0.001$).

The hearts induced with Bretschneider HTP solution showed an approximate threefold increase in resistance compared with the other three solutions (Figure 5D).

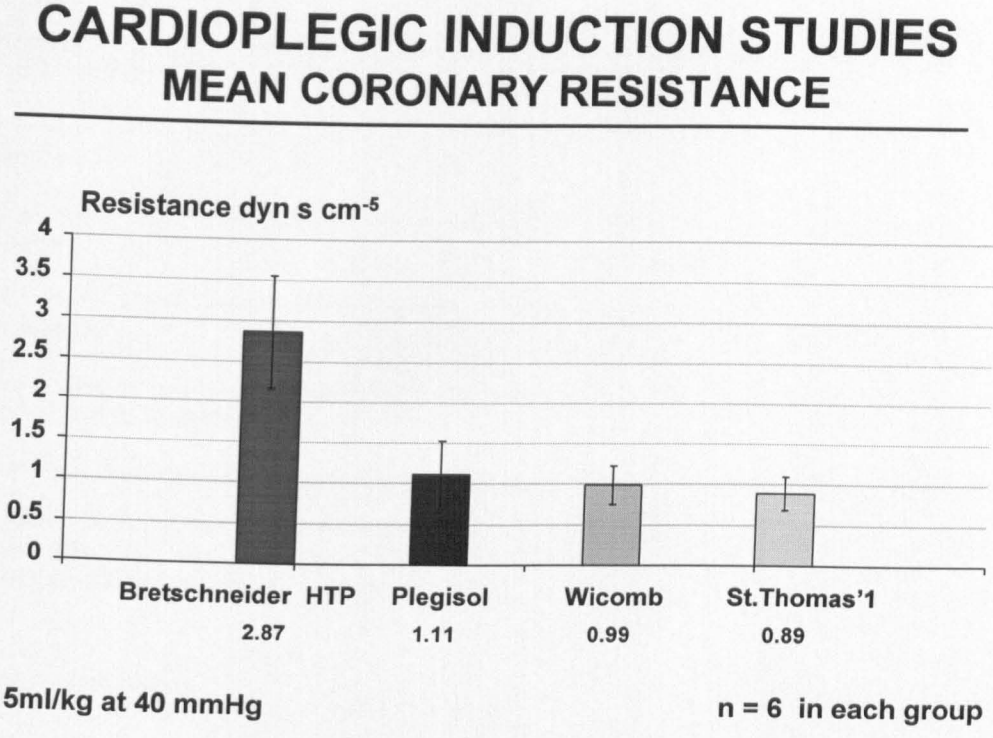


Figure 5D Histogram showing the differences in coronary resistance between the four solutions. As with time to arrest, the hearts arrested with HTP solution showed a much higher resistance. St.Thomas' 1 showed a significantly lower resistance compared with Plegisol.

The mean coronary resistance for the four solutions are ranked as follows:

St. Thomas' No.1< Wicomb < Plegisol << *Bretschneider HTP*

TABLE 5.2

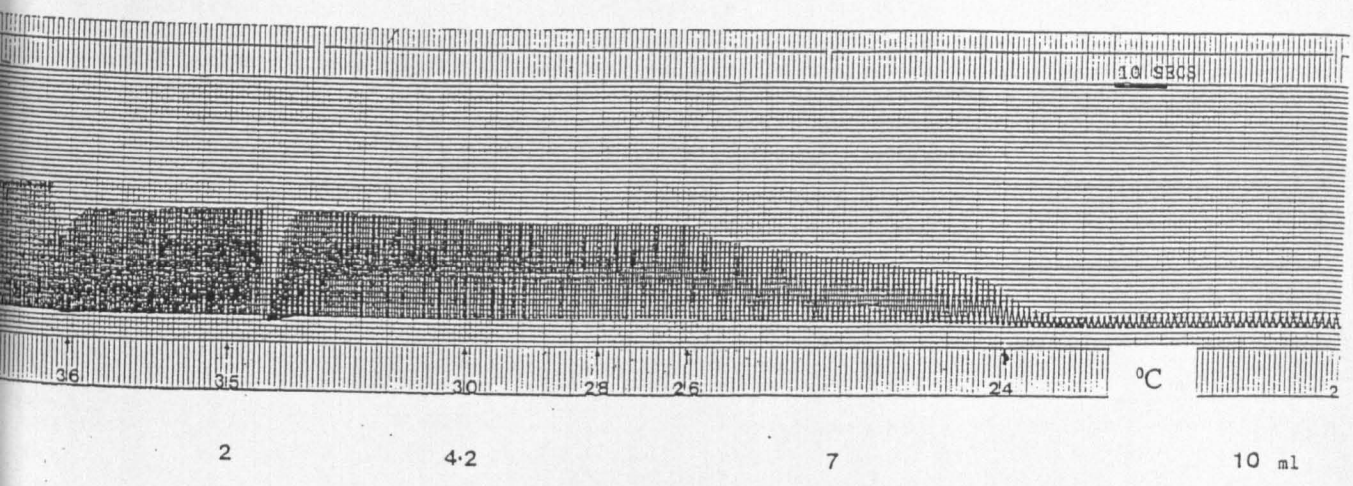
SOLUTION	T_{ARR} seconds	C_R dyn s cm^{-5}
Bretschneider HTP	78	106.4
Plegisol	28	40.7
Wicomb	24	33.3
St. Thomas' 1	17	36.5
STANDARD DEVIATION	3.1	12.6

The above Table shows the large differences between the solutions with respect to their efficacy at cardioplegic induction. Whilst the major effect would seem to be flow related their are also differences related to the chemical composition.

Hypothermia Alone

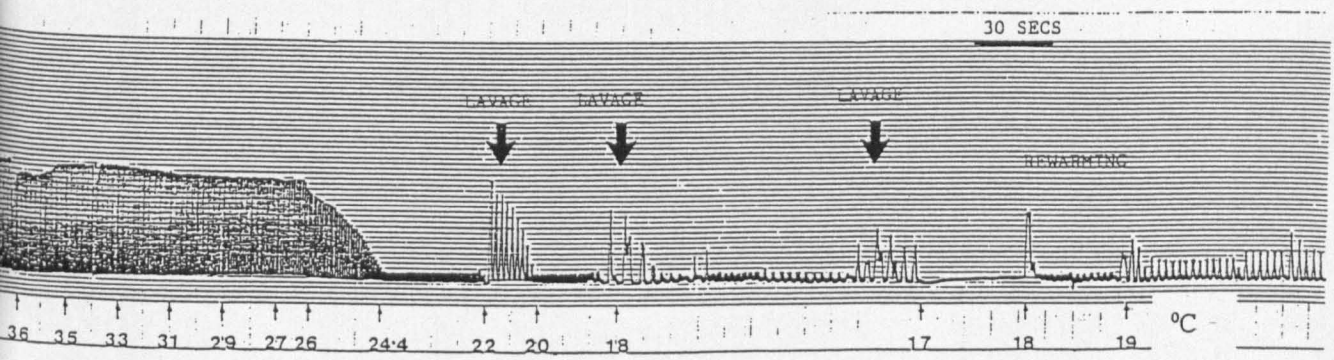
Using Kreb's solution delivered at 4°C and at a pressure of 40 mmHg, it was established that guinea-pig hearts undergo a series of stepwise reductions in electromechanical activity, at 26°C, 24°C and final standstill at 17°C. The time taken to cool these hearts, by coronary perfusion alone, is a function of coronary resistance, which was approximately 3 times as high as that of the high potassium solutions. At a delivery pressure of 40 mmHg it was not possible to cool these hearts below 23°C without external cooling (Figure 5E).

Effects of Hypothermia Alone



Vertical axis 20mm = 100mmHg

Volume of Kreb's Solution



Vertical axis 20mm = 100mmHg

Figure 5E Using hypothermia alone, as an inductive agent, demonstrates stepwise changes in activity at approximately 26°C, 24°C and standstill at 17°C.(Upper trace). In the lower trace 4°C Kreb's solution is administered at 40 mmHg as an inductive agent. However, the coronary resistance is too high to allow effective cooling, without external lavage (Lower Trace).

^{31}P-NMR Spectroscopy

Spectral analysis of these hearts, 20 minutes after arrest, showed normal levels of high energy phosphates in the Wicomb perfused group, but significantly reduced levels in all of the other solutions. Whilst statistical analysis was not performed on this data, because of the small numbers, the qualitative differences seen in the spectra are impressive (Figure 5F). Ranking for ATP and Phosphocreatine were:

Wicomb > Plegisol > St Thomas' No. 1>> Bretschneider HTP

There were also differences in intra-cellular pH measured at 20 minutes. These were:

St.Thomas'No. 1 **7.35**. Plegisol **7.30**.

Wicomb **7.25**. Bretschneider HTP **7.0**

In these spectra the chemical shifts are assigned in parts per million (ppm) relative to the phospocreatine (PCr) signal, which is assigned a value of 0 ppm. Signals are assigned to the beta, alpha and gamma phosphates of ATP, PCr and inorganic phosphate (Pi). The peaks at 3 ppm and at 6 to 7 ppm are from phosphodiesters and phosphomonoesters, respectively.

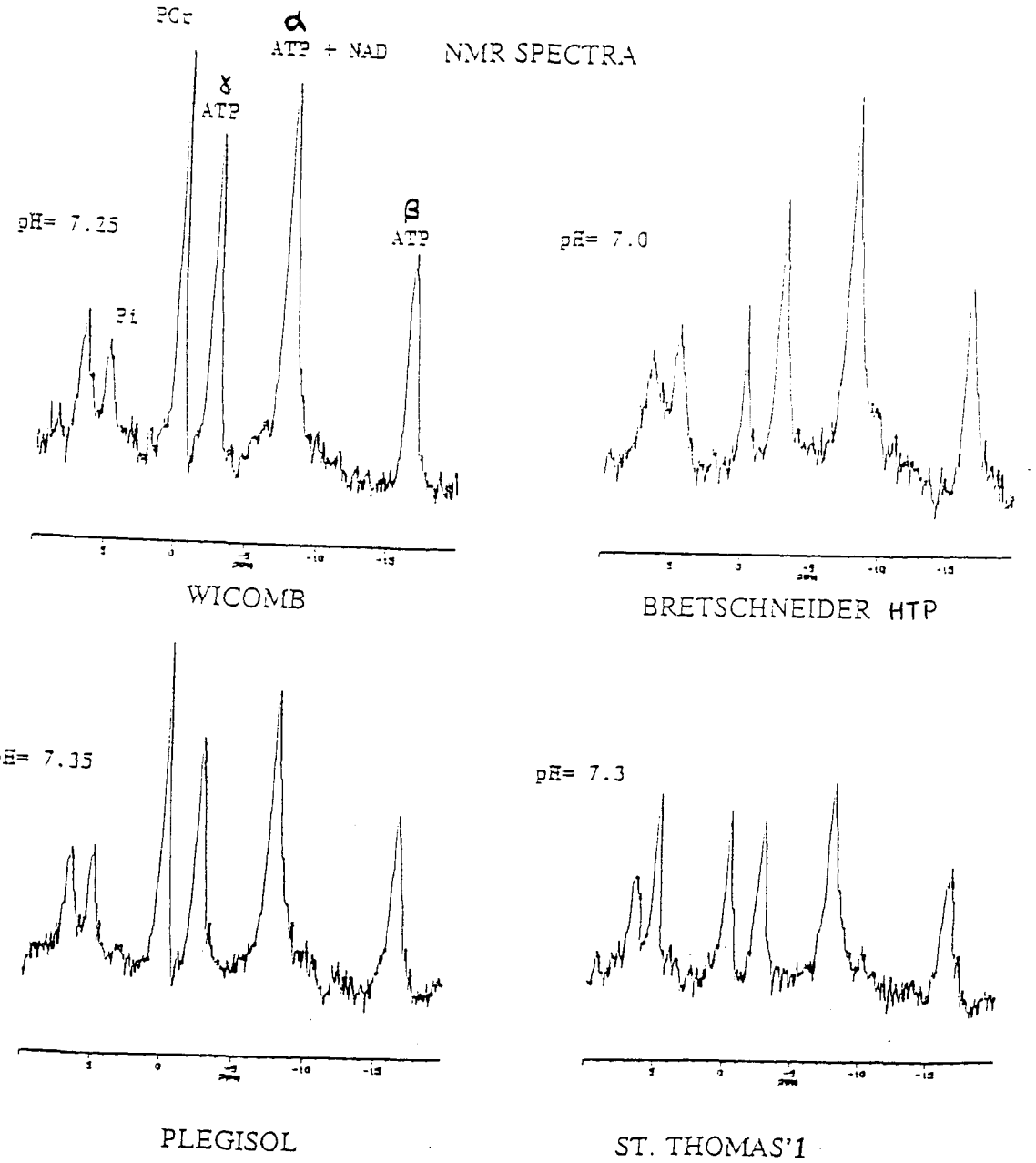


Figure 5F The above NMR spectra were obtained following 20 minutes of storage in each solution. Wicomb solution exhibited the best preservation of both phosphocreatine and ATP with St Thomas' 1 and Bretschneider HTP solutions showing early degradation.

Pi = Inorganic Phosphate
ATP = Adenosine Triphosphate
PCr = Phosphocreatine
NAD = Nicotinamide Adenosine Dinucleotide

5.4 DISCUSSION

In the course of cardiac transplantation, there are five major stages which impact on donor heart function; initial donor management, cardioplegic induction, cold storage, global ischaemia during implantation, and reperfusion. Hyperkalaemic, hypothermic cardiac arrest has remained the cornerstone of all modern methods of cardioplegia, despite an ongoing debate over many other aspects of composition, temperature and mode of delivery. However, none of the cardioplegic solutions in current clinical use was expressly designed for donor heart preservation.

In biological systems the strong depressant effect of cooling is often expressed by the van't Hoff rule, which relates the rate of a particular reaction at one temperature (T_1) to that of the same reaction at a temperature difference of 10° C - the so-called Q_{10} relationship[192].

$$Q_{10} = \text{rate at } T_1 + 10^{\circ}C/\text{rate at } T_1$$

For many biological reactions the Q_{10} values are 2 or more. It is on this powerful relationship that the beneficial effects of cooling tissues for clinical preservation are based. However, this depressant effect is not selective and those processes responsible for maintaining the constituent tissues are also affected.

When the logarithm of the rate of a simple chemical reaction is plotted as a function of the reciprocal of absolute temperature (Arrhenius plot), a straight line relationship is produced, the slope of which depends on the activation energy of the reaction[193]. In typical biological reactions in tissues, however, each step will almost certainly have a different activation energy, making the overall effect unpredictable, and discontinuities have frequently been reported in plots of biological processes[194].

However, as working methods for quantifying the effects of temperature on biological process, both the Arrhenius and van't Hoff relationships have proved invaluable.

The effects of cooling on cellular metabolism and membrane transport are therefore complex[195,196]. These complex effects extend to changes in physical properties brought about by cold, as exemplified in the lipid phase changes in membranes[197] and deleterious effects on the cytoskeleton[198,199]. Most biological reactions have a structural-functional interrelationship which can be disrupted by spatial changes, sometimes leading not only to a loss of function but the possible creation of aberrant processes as in the production of oxygen free radicals[200].

Cooling influences both the energy available for cellular activities such as ion pumping and protein synthesis, but also limits the rate of energy production, such as high energy phosphate synthesis[201]. Inhibition of active and facilitated membrane transport systems reduces the uptake of substrate and causes ion distribution to be disturbed[202]. This effect extends to the maintenance of the intracellular ionic gradient which, at normothermia, results from a balance between the passive flux of ions down their electrochemical gradients and the activity of energy-dependent ion pumps working against gradients[202]. The net result is that at lower temperatures the rate of ion pumping is usually unable to match the rate of passive ion fluxes and, owing to the charge carried by intracellular proteins, there is a net gain of sodium and chloride ions within the cell, leading to cellular oedema[207]. This means that there is a need to understand how cooling affects integrated cellular metabolism and select the best compromise. This has not generally been the case in clinical organ preservation.

In the experiments described in this chapter, the coronary resistances developed as a consequence of perfusing guinea pig hearts at a constant pressure were shown to vary threefold between solutions. The reasons for this wide disparity are probably related to potassium mediated vascular tone changes rather than viscosity differences, since Wicomb solution was the most viscous of the solutions, and Bretschneider HTP has a substantially lower potassium content. Resistance curves (at a range of perfusion pressures) were not contructed as this was not pertinent to the experiments described, in which it was necessary to keep as many of the conditions as possible, constant.

The use of a cardioplegic solution for intra-operative myocardial protection, has some significant differences from the application for donor heart storage. Induction usually takes place after active body cooling has been initiated via the cardio-pulmonary bypass machine (CPB) and likewise the CPB crystalloid prime has reduced the haematocrit to around 30%. Mean arterial pressures are usually low and the heart has usually had a period during which full perfusion with cold oxygenated blood has coincided with a period of cardiac decompression. Following cardioplegic induction, whether antegrade, retrograde, warm or cold, the cardioplegia will be eliminated from the coronary vascular bed by the collateral circulation, within 10-15 minutes.

In this chapter the differences between four representative solutions were investigated with regard to their efficacy in achieving rapid diastolic arrest, with minimal substrate consumption (as measured by NMR), in a setting representative of donor heart retrieval. There was more than a fourfold difference in time to arrest between the most rapid (St. Thomas' 1) and the slowest (Bretschneider HTP) inductive solution. Possibly more importantly, NMR indicated better high energy phosphate levels in both Wicomb

and Plegisol solution, after 20 minutes of hypothermic storage. The use of NMR is a particularly useful method of monitoring preservation since the intracellular environment can be monitored for both high-energy phosphate depletion and the accumulation of acid metabolites. A few preliminary experiments also confirmed that it would be possible to carry out the low-flow perfusion within the magnet.

Whilst ATP levels are not the only factor controlling viability, high energy phosphates do play a central role in active transport processes[204]. This view is underlined by the increased use of NMR as a powerful technique in non-invasive studies of energy metabolism during both warm and cold ischaemia[205-208].

Bretschneider HTP solution was chosen to represent the middle European solutions which follow an *intracellular* composition together with histidine, which is a more effective buffer at low temperatures. St Thomas' No.1 solution represents the most popular generic group of *extracellular* solutions and Plegisol is a more recent variant in which the ionised calcium content has been halved and the potassium content reduced. Wicomb solution is an experimental dextrose based solution, containing lower potassium and magnesium levels, together with the calcium antagonist Verapamil. Insulin is included to facilitate the anaerobic metabolism of dextrose. The solution was designed specifically for donor heart preservation by the Cape Town group[21].

The results show Bretschneider HTP solution to be particularly ineffective for donor heart preservation, in this model. However, since these experiments were conducted the solution has been modified (designated HTK)[209] and the delivery method considerably altered[210]; perfused volume has been increased to 36 ml/kg and the solution is delivered first in an oxygenated form, followed by a non oxygenated

solution. A recent retrospective multicentre study suggests that HTK now offers similar protection to other solutions in common usage[211].

Lowering the calcium content and reducing the potassium concentration may make Plegisol a more effective solution than St.Thomas' 1. The hyperkinetic phase prior to arrest is avoided but this would seem to be offset by the more rapid onset of diastole in the case of St.Thomas' 1, as evidenced by the NMR data. Surprisingly, normal pH was better maintained in the St.Thomas' group despite the theoretical superiority of histidine buffering in Bretschneider HTP. In an NMR study of the effects of sodium and calcium levels on ATP, Pernot found that calcium levels in the 1 - 0.25 mmol range gave best substrate preservation but that this was linked to maintaining high levels of external sodium, since a rise in the potassium:sodium ratio enhances calcium influx to the cytosol and hence energy consumption[212]. In a study comparing four similar solutions, English found that whilst initial substrate levels were better preserved with the *extracellular* solutions, as was pH, Bretschneider HTP solution was superior to St.Thomas' 1 after 4 hours preservation (the rates of degradation were different). However, a similar dextrose based solution to Wicomb (designated CP5) was found to be more effective, overall, after 24 hours storage[22]. In addition, these hearts were perfused with much higher volumes of cardioplegia (60 ml/kg), a feature which seems to be important for the Bretschneider compositions.

5.6 CONCLUSIONS

This study indicates that of the solutions tested, the dextrose based Wicomb solution provides the most effective method of cold cardioplegic arrest. This chapter does not seek to address whether or not a solution which provides effective induction is also capable of providing the optimal protection required for long term global ischaemia for

donor heart preservation applications. This question is investigated in the following Chapters.

CHAPTER 6

DELIVERY METHOD AND OXYGENATION

6.1 BACKGROUND

In the previous chapter the relative efficacies of a range of generically representative cardioplegic solutions were investigated with respect to cardioplegic induction. This chapter explores one aspect of functional preservation, during the global ischaemia which accompanies donor heart storage.

Oxygenated cardioplegia has recently been employed with both blood based[213] and crystalloid solutions[214], as a means of providing better myocardial protection during routine cardiac surgery. This has resulted from the knowledge that whilst hypothermia and hyperkalaemic cardioplegia may greatly reduce myocardial oxygen demand, there is still a finite oxygen requirement of some 0.5 $ml.min^{-1}.100g^{-1}$ of muscle at $4^{o}C$[215]. If this demand is not met, anaerobic metabolism can support myocardial viability for a period, limited by; the existance of available substrate, a reduction in high-energy phosphates, an increase in acidosis and the accumulation of toxins and inhibitory metabolites[216]. Which of these is most important, is unclear. It may be sufficient to remove waste products with an appropriate flow, without attempting to meet the aerobic oxygen and substrate demand. On the other hand, oxygen and substrate delivery may be paramount.

During routine cardiac surgery cardioplegia may be delivered by single flush[186], intermittent perfusion[217] or by continuous perfusion[218]. In donor heart preservation

single flush followed by hypothermic storage is almost exclusively used (Appendix A), mostly for logistical reasons. In this chapter the interactions between delivery method and level of oxygenation, using a single crystalloid cardioplegic base solution, was investigated. St Thomas' 1 cardioplegia was chosen as being representative of the most commonly used generic hyperkalaemic solutions, that is, a balanced electrolyte solution with increased potassium in the 16 to 20 mmol range.

Since it is unclear what the relationship between perfusion and oxygen delivery is, these experiments were conducted in two phases; the first examined the effects of increased oxygen supply by three different delivery methods, and the second examined the effects of keeping the oxygen delivery constant whilst changing the rate of perfusion.

6.2 MATERIALS AND METHODS

The isolated perfused working heart model, as described in preceding chapters, was used for these studies. Guinea pig hearts were arrested with 15 ml/kg of the cardioplegic solution under test, following control function curves. The work was carried out in two phases; Study 2 was designed to further investigate the continuous perfusion mode of delivery.

Study 1

Seventy two isolated guinea-pig hearts were randomised into 9 groups and studied before and after arrest and storage with a standard cardioplegic solution (St.Thomas' 1) for three hours at 4°C.

Three methods of cardioplegic delivery were used:

1. Continuous low-flow perfusion (4 ml/hr) **(CP)**
2. Intermittent perfusion (1 ml/min for 2 minutes every 30 minutes) **(IP)**
3. Single flush (15 ml/kg) **(SF)**

Solutions with three different oxygen tensions were used:

1. pO_2 of 240 mmHg. **(Low)**
2. pO_2 of 560 mmHg. **(Medium)**
3. pO_2 of 950 mmHg. **(High)**

These levels of oxygenation were chosen to represent typical levels for atmospherically equilibrated solutions **(Low)**, bubble oxygenated solutions **(Medium)**, and hyperbarically oxygenated solutions **(High)**.

Oxygenation was achieved by prewarming followed by variable hyperbaria, a method which was developed by the author and validated to provide crystalloid solutions with stable predetermined oxygen contents[219](Bibliography D).

Study 2

Following this first study a further 42 hearts were studied; 24 Constant Flow (as in Study 1), and 18 Adjusted Flow, in which the flow rate was adjusted for oxygen content, so as to maintain a constant oxygen delivery. These groups were designated **CF** and **AF** respectively. The **CF** group was equivalent to the **CP** group from Study 1. Each group was further sub-divided according to the pO_2 of the solution, as before.

The number of experiments in each treatment regime is shown in Table 6.1.

TABLE 6.1

DELIVERY METHOD

	STUDY 1			STUDY 2	
	CP	**IP**	**SF**	**CF**	**AF**
LOW	8*	8*	8*	8	6
MEDIUM	8	8	8	8	6
HIGH	8	8	8	8	6

Delivery methods and oxygenation were randomised.
* One heart in each of these groups failed to eject against the 50 mmHg afterload, post storage, and was excluded from the analysis.

Oxygenated cardioplegic flow rates for the **CP** group were determined by theoretical calculation of myocardial oxygen consumption based on 0.15 $0_2.min^{-1}.100g^{-1}$ [176,220]. This gives a calculated consumption of 0.18 mls of oxygen/hr for a hyperkalaemically arrested 2.0g guinea pig heart at 4°C (Figure 6A). The flow required to meet this demand using the **High** oxygen solution (950 mmHg) would be 3.3 ml/hr. (Figure 6B).

DETERMINATION OF OXYGEN DELIVERY

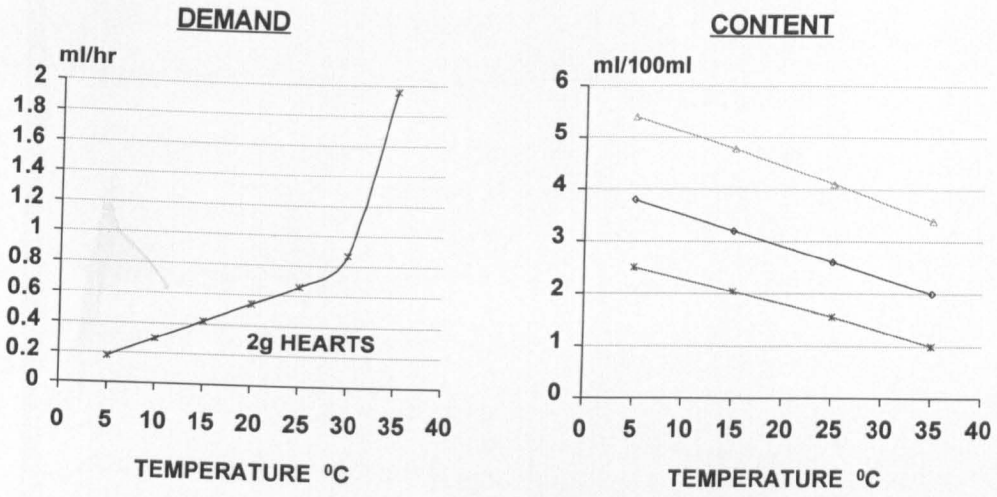


Figure 6A The effect of temperature on the mean myocardial oxygen demand for hyperkalaemically arrested hearts, and the oxygen content of crystalloid solutions at the three gas tensions under investigation.
The value for available oxygen dissolved in asanguinous solutions, were derived from solubility constants for oxygen, adjusted for temperature[221].

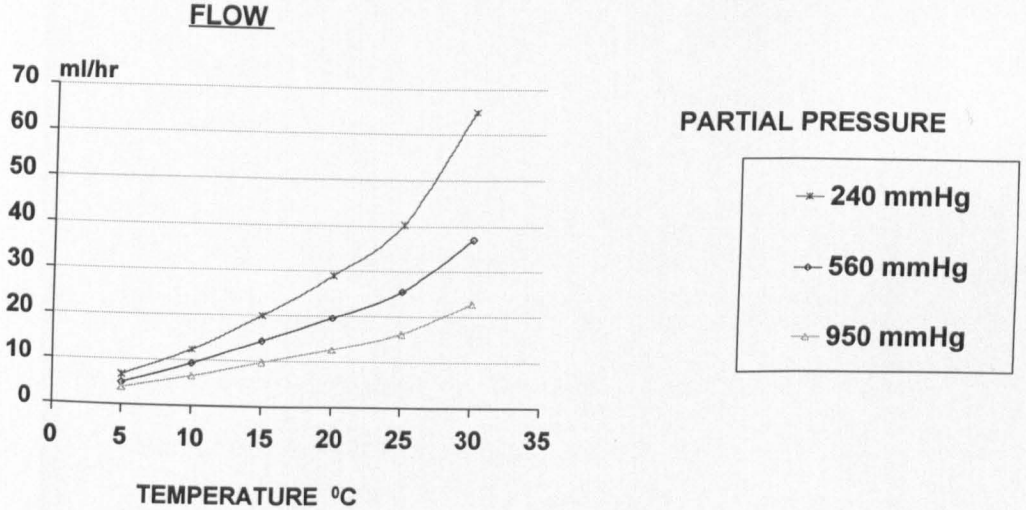


Figure 6B With a knowledge of the oxygen demand and the oxygen content, with respect to oxygen tension, the required flow curves can be plotted for the three oxygenated solutions under investigation. These flows are calculated to meet the demands of a 2g heart.

These estimations were checked on three hearts by measuring coronary effluent pO_2 whilst reducing perfusion flow from 6 ml/hr by 0.5 ml/hr every 30 minutes. There was a sharp decrease in coronary sinus pO_2 between 3.5 and 3.0 ml/hr, suggesting that oxygen consumption exceeded supply between these flows. A flow of 4 ml/hr was therefore chosen for the CP group in Study 1.

For Study 2 the perfusion flows were adjusted to maintain the same oxygen delivery, in Group AF. The individual flows for the three levels of oxygenation were:

LOW = 8 ml/hr **MEDIUM** = 6 ml/hr **HIGH** = 4 ml/hr

Analysis

Function curves were produced for each heart pre and post storage.

Each curve was summarised by calculating:

1. Mean stroke volume at LAP 5 to 25 cmH_2O (3.7 to 18.5 mmHg)
2. Maximum stroke volume.
3. LAP where maximum stroke volume occurs (LAP_{max}).

Each of these parameters from the post storage function curve was analysed separately by analysis of variance (ANOVA), taking into account the delivery method and the amount of oxygenation. The respective parameter value from the pre storage function curve was used as a covariate.

Cardiac output was not analysed as it was highly correlated with Stroke Volume, and the latter was used in this study so as to remove the effects of heart rate. These hearts were not paced.

6.3 RESULTS

Study 1

Function curves were constructed for each delivery mode and level of oxygenation.

Figures 6C, 6D and 6E illustrate the effects of different delivery methods combined with the three levels of oxygenation.

SINGLE FLUSH DELIVERY
EFFECTS OF OXYGENATION

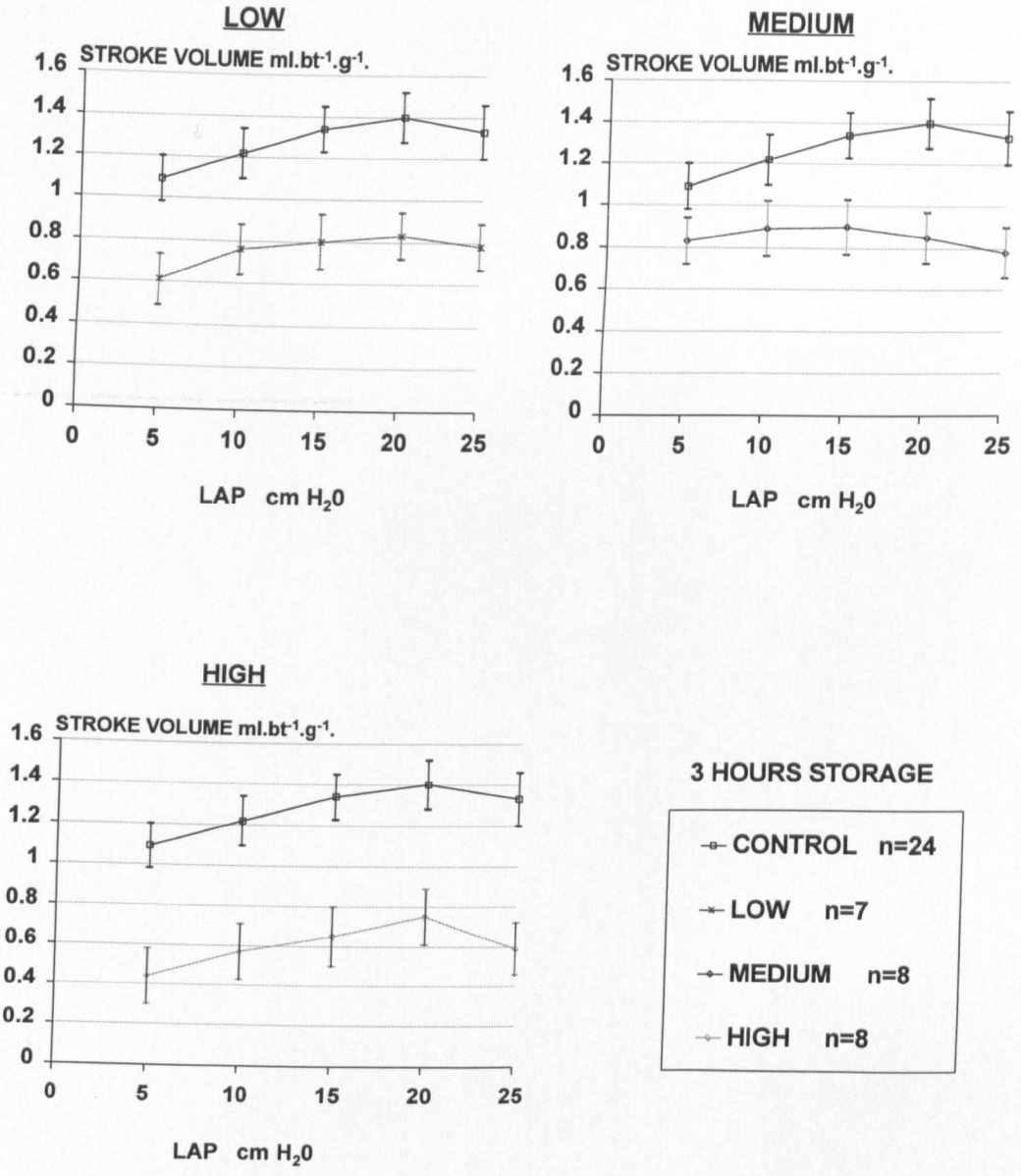


Figure 6C The Stroke Volume is plotted for each 5 cm increase in preload post 3 hour storage following single flush preservation. The pooled pre-storage curve is shown for comparison. All post storage curves show an approximate 40% loss of function with the MEDIUM group giving the best results. (1 cm preload = 0.74 mmHg)

INTERMITTENT DELIVERY
EFFECTS OF OXYGENATION

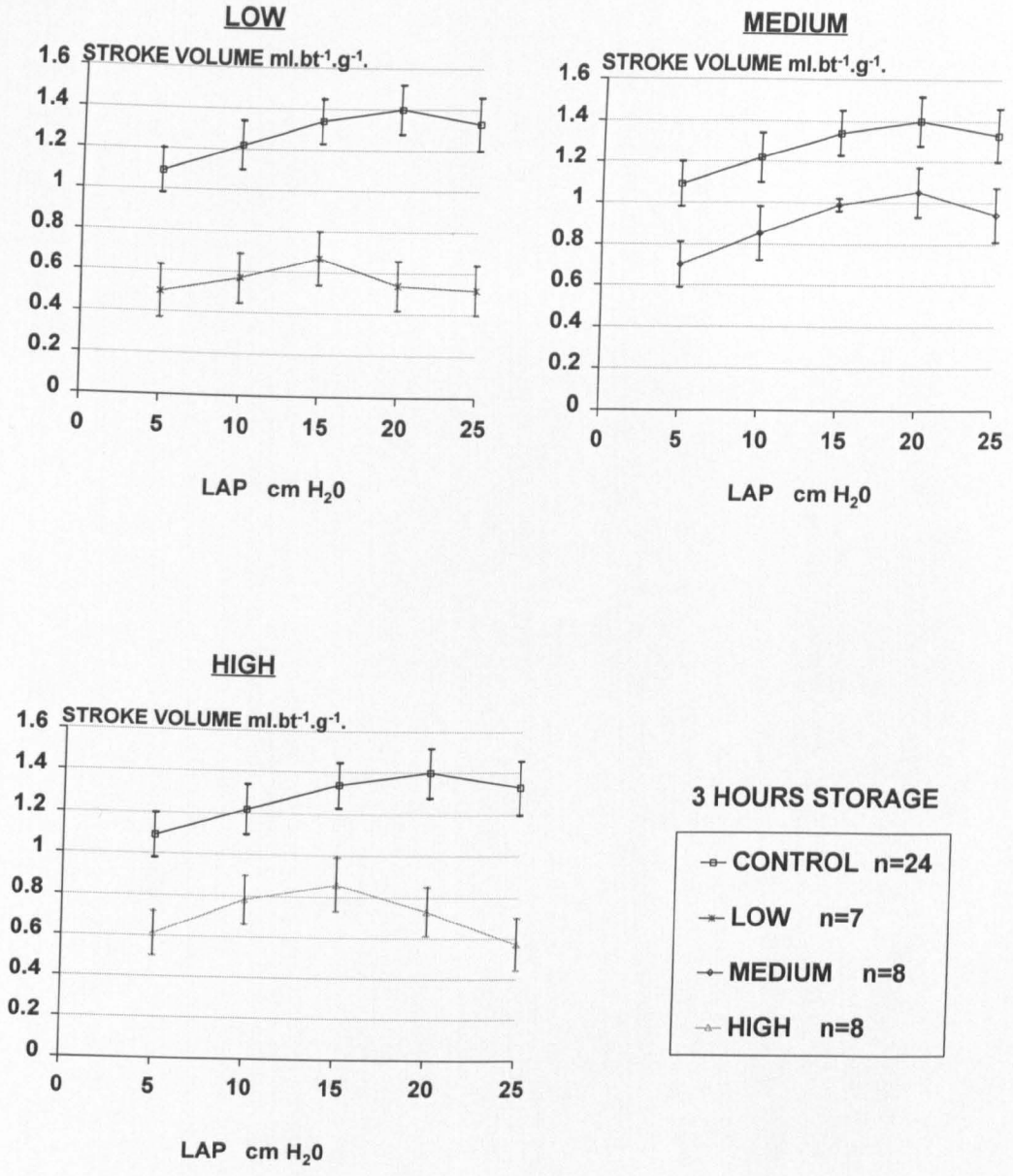


Figure 6D With Intermittent delivery there is a more pronounced difference between the post storage curves, depending on oxygenation. The LOW group only achieves approximately 50% of pre-storage function.(1 cm preload = 0.74 mmHg)

CONTINUOUS DELIVERY
EFFECTS OF OXYGENATION

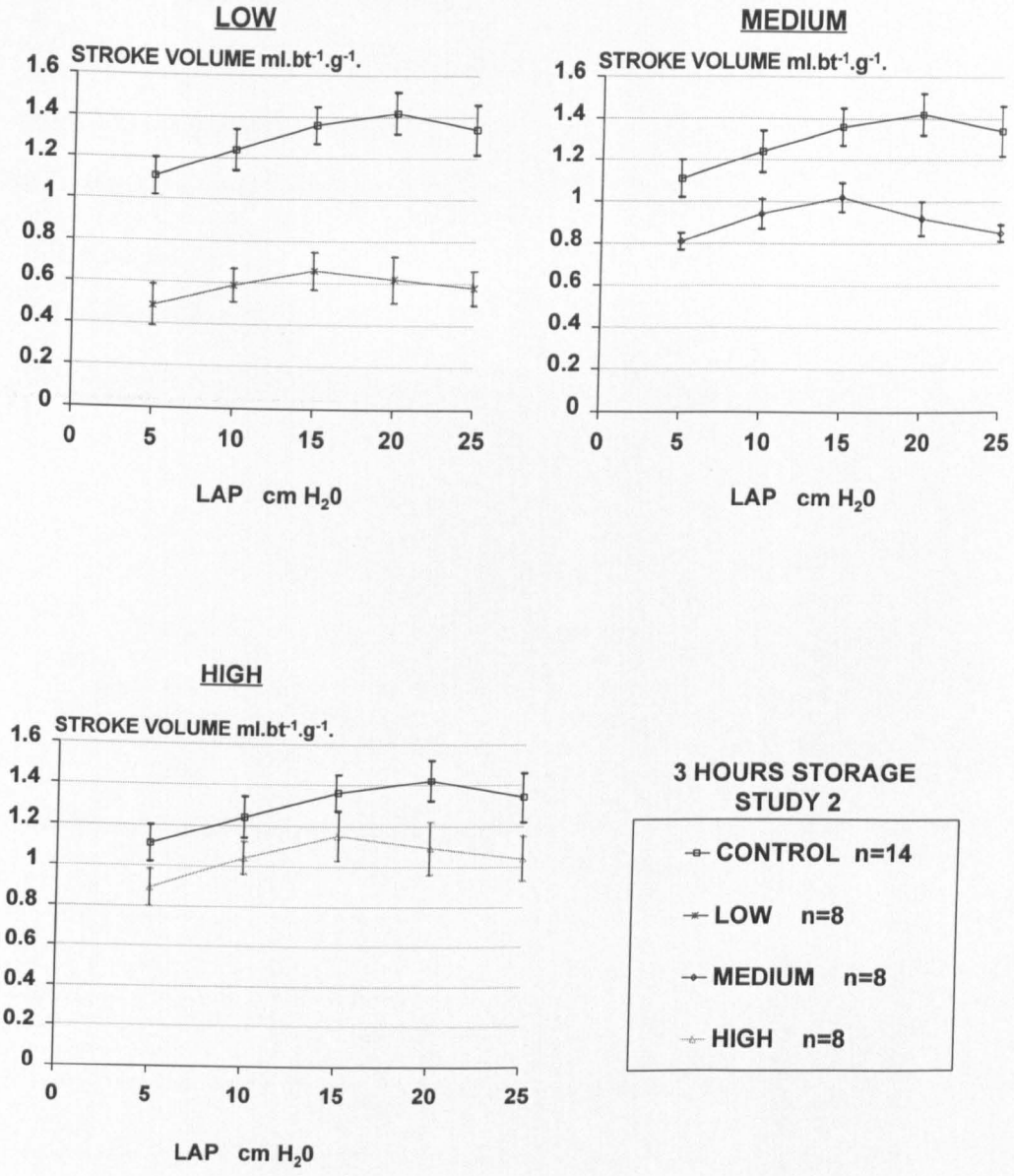


Figure 6E Continuous delivery shows a marked difference between the LOW oxygen group (< 50% of pre-storage) and the other two groups which were able to maintain about 80% of pre-storage function.(1 cm preload = 0.74 mmHg)

Mean Stroke Volume

There was a statistically significant interaction between the effects of the different methods of delivery and the amount of oxygen on the mean Stroke Volume ($p = 0.002$). For the *Continuous* perfusion delivery method, Stroke Volume increases with increasing oxygenation. However, for the *Intermittent* and *Single Flush* methods, the highest mean Stroke Volumes were observed at a pO_2 of 560 mmHg.

Continuous perfusion (**CP**) with oxygenation at 950 mmHg produced the highest mean Stroke Volume of all methods in Study 1 (0.94 ml/bt) (Table 6.2).

TABLE 6.2

MEAN STROKE VOLUME ($ml.bt^{-1}.g^{-1}$)

DELIVERY METHODS

	Study 1			**Study 2**	
Oxygen	CP	IP	SF	CF	AF
LOW	0.52	0.60	0.72	0.57	0.69
MEDIUM	0.86	0.90	0.78	0.92	0.78
HIGH	0.94	0.71	0.61	0.97	0.95
SD	0.0489			0.0557	

Maximum Stroke Volume

The results for maximum Stroke Volume were very similar to that of the mean. There was a statistically significant interaction between the effects of the different methods of delivery and the amount of oxygen ($p = 0.004$). Maximum Stroke Volume increased

with the amount of oxygenation for the *Continuous* method. However, for the *Single Flush* and *Intermittent* methods the highest maximum Stroke Volumes were observed at 560 mmHg (0.90 ml and 1.05 ml/bt, respectively).

Continuous perfusion (**CP**) with oxygenation at 950 mmHg produced the highest maximum Stroke Volume of all methods (1.12 ml/bt) (Table 6.3).

TABLE 6.3

MAXIMUM STROKE VOLUME ($ml.bt^{-1}.g^{-1}$)

DELIVERY METHODS

	Study 1			**Study 2**	
Oxygen	CP	IP	SF	CF	AF
LOW	0.62	0.67	0.83	0.66	0.79
MEDIUM	0.97	1.05	0.90	1.02	0.91
HIGH	1.12	0.86	0.75	1.15	1.09
SD	0.0555			0.0633	

LAP_{MAX}

There were no statistically significant interactions between the effects of the different methods of delivery and the amount of oxygen on the pre-load at which maximum Stroke Volume was obtained ($p > 0.05$). This suggests that the trend in the effect of oxygenation was similar for all three methods of delivery (Table 6.4).

TABLE 6.4

LAP_{max} (mmHg)

DELIVERY METHODS

Oxygen	CP	IP	SF	CF	AF
LOW	13.3	11.1	12.6	11.8	10.4
MEDIUM	11.1	11.8	11.1	10.4	13.3
HIGH	12.6	11.1	14.1	11.8	11.8
SD	2.8			2.7	

CONTINUOUS DELIVERY
EFFECTS OF OXYGENATION

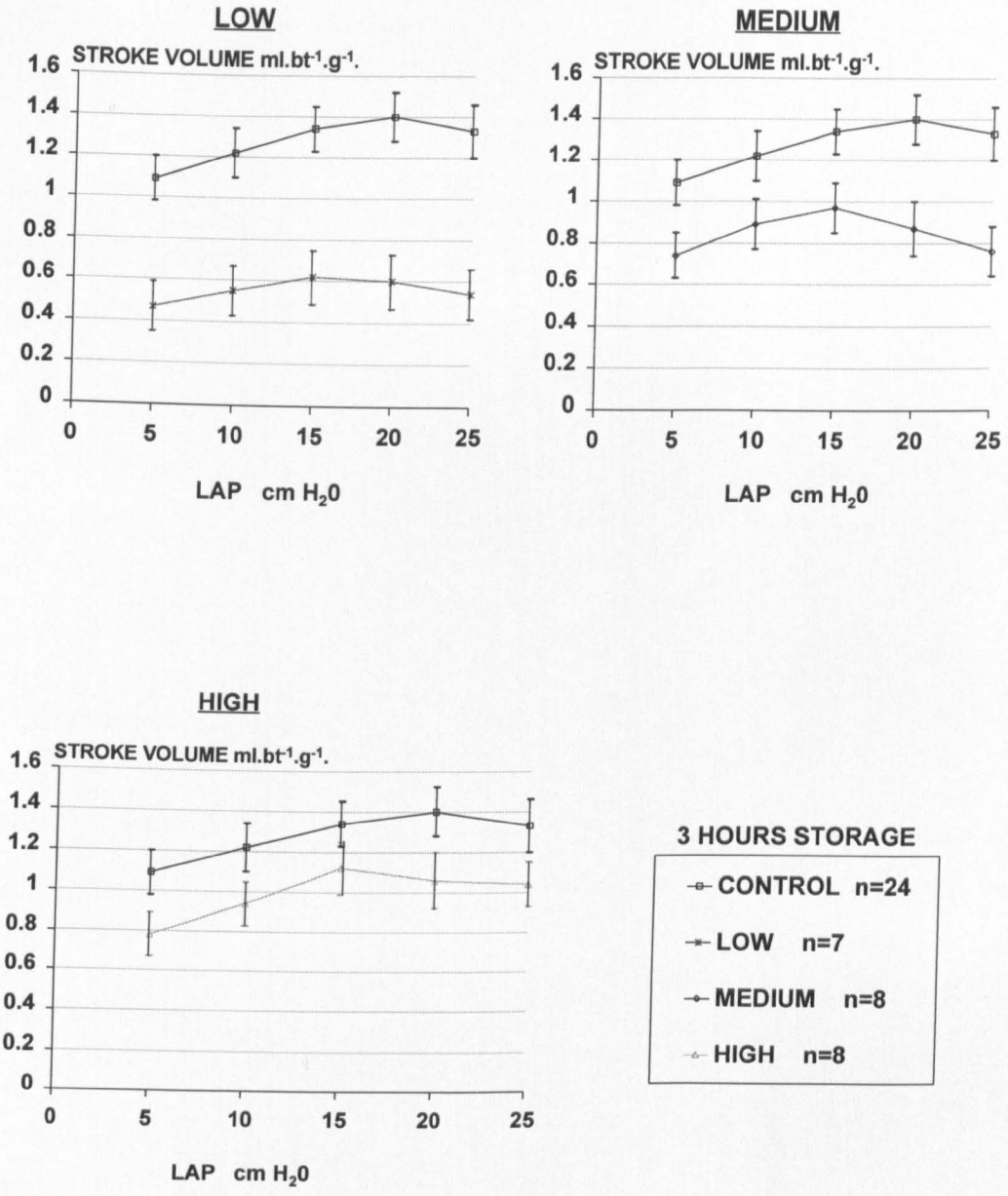


Figure 6F This group was treated in exactly the same way as the continuous group in Figure 6E but in Study 2, conducted at a later time. The results parallel those in the earlier study. (1cm preload = 0.74 mmHg)

FLOW ADJUSTED DELIVERY
EFFECTS OF OXYGENATION

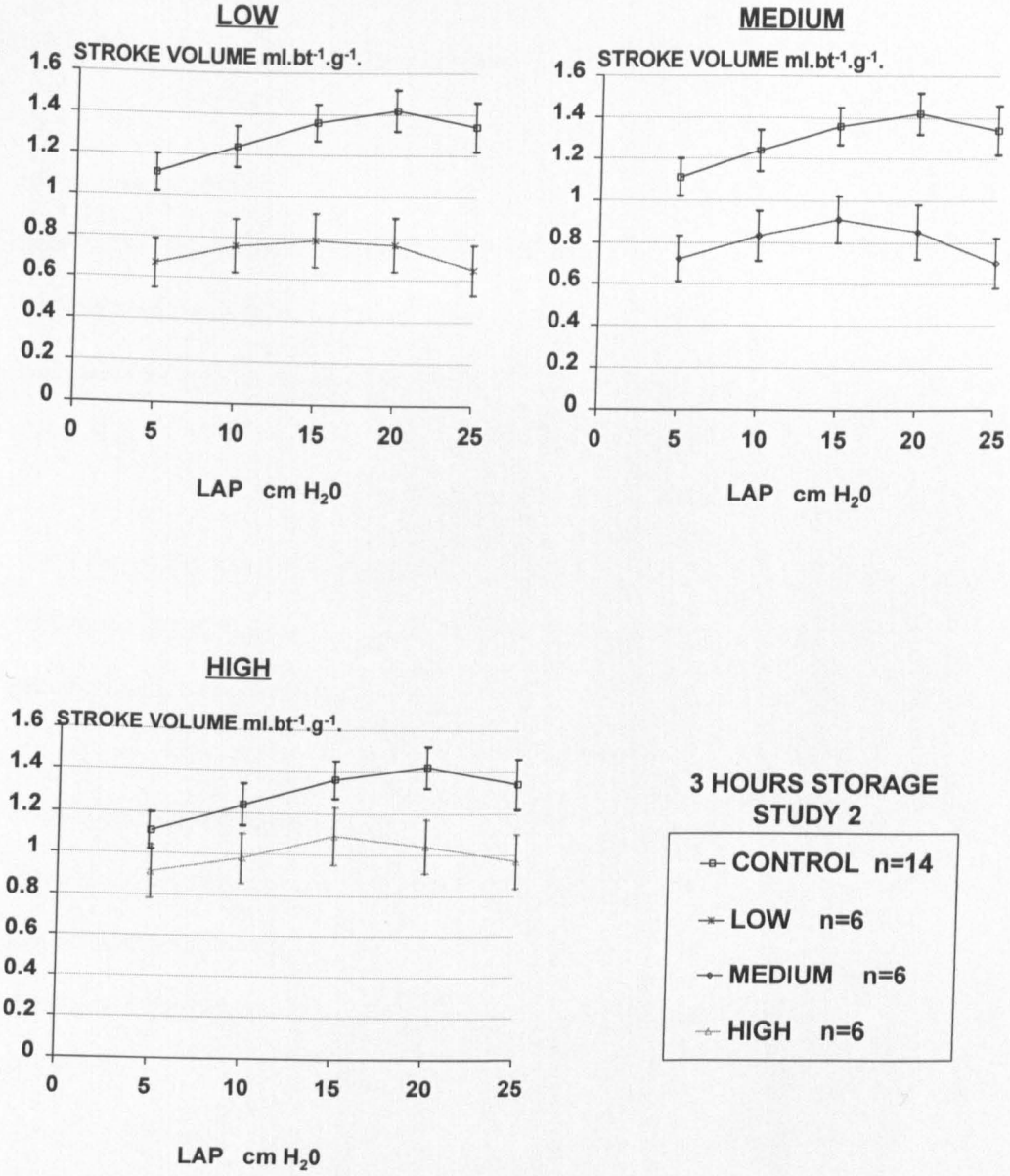


Figure 6G In this group the continuous flow was adjusted, according to oxygen content, in order to deliver the same oxygen supply in all three cases. Functional preservation can be seen to improve with increasing oxygen *content*.
(1cm preload = 0.74 mmHg).

Study 2

In the second group of experiments the impact of providing equivalent amounts of oxygen by different delivery methods, was compared. Function curves of the two delivery methods were constructed as illustrated in Figures 6F and 6G.

Mean Stroke Volume

There was no statistically significant **interaction** between the different methods of delivery and the different levels of oxygen on the mean Stroke Volume ($p = 0.313$). Nor was there a statistically significant effect between the different delivery methods **alone** ($p= 0.832$).

However, there was a statistically significant effect between the different **levels** of oxygen on the mean Stroke Volume ($p< 0.001$). The mean Stroke Volume increases with increasing oxygen level (Table 6.2).

Maximum Stroke Volume

There was no statistically significant **interaction** between the different methods of delivery and the different levels of oxygen on the mean Stroke Volume ($p = 0.367$). Nor was there a statistically significant effect between the different delivery methods **alone** ($p= 0.896$).

There was a statistically significant effect between the different **levels** of oxygen on the mean Stroke Volume ($p< 0.001$). The mean Stroke Volume increases with increasing oxygen level.

The delivery method with constant flow and altered oxygen content (**CF**), with **HIGH** (950 mmHg) oxygen tension produced the highest maximum Stroke Volume (1.15 ml/bt), as in Study 1 (Table 6.3).

LAP $_{max}$

There was a statistically significant interactive effect between the different delivery methods and the levels of oxygen on the pre-load at which maximum Stroke Volume was obtained ($p = 0.032$). This suggests that the effect of oxygen was dissimilar for both delivery methods. For the constant flow mode of delivery (**CF**), the lowest LAP_{max} was with the **MEDIUM** (560 mmHg) level of oxygen, and the highest at the **LOW** (240 mmHg) level of oxygen. For the constant level mode of delivery (**AF**), the lowest LAP_{max} was obtained with the **LOW** (240 mmHg) level of oxygen, and the highest at the **MEDIUM** (560 mmHg) level of oxygen (Table 6.4).

6.4 SUMMARY

In summary, the method of cardioplegia delivery was shown to be interactive with the oxygen content of the solution such that **High** oxygen is beneficial with the continuous methods of delivery, whilst **Medium** oxygenation gives improved functional preservation with all methods of delivery. Perfusion with **Low** levels of oxygen seems to be detrimental, whilst perfusion with **High** levels of oxygenation gives the best results. Figure 6H summarises the percentage recovery of maximum stroke volume for each method, with respect to control values.

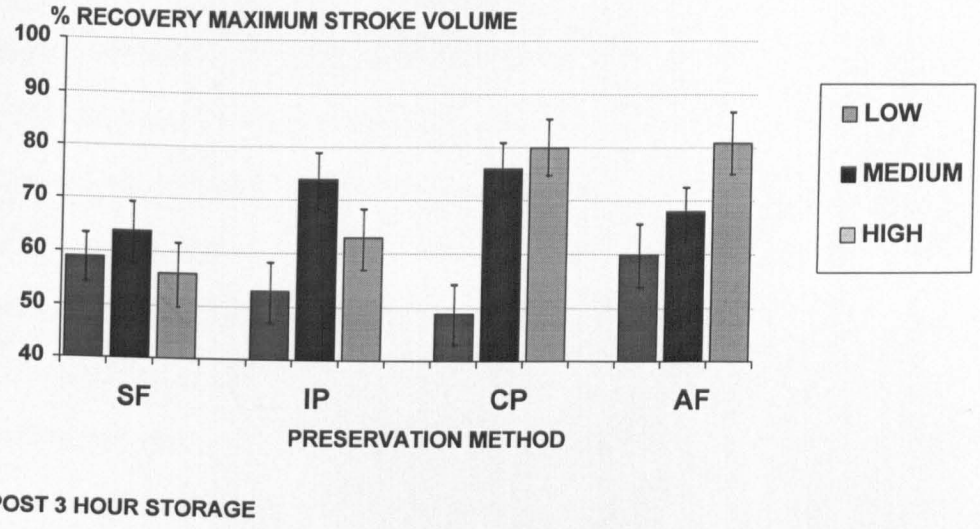


Figure 6H The percentage changes (from pre-storage controls) in maximum Stroke Volume are illustrated across the treatment groups. It is clear that function is increasingly better preserved with increasing oxygen content, in the continuous perfusion methods but medium levels of oxygen give better preservation with non-continuous delivery.

In Study 2, which looks more carefully at the continuous methods of delivery decreasing the oxygen *level* is shown to be detrimental (lower means, maximums and flatter curves) regardless of delivery method. Conversely, ensuring sufficient oxygen to support aerobic metabolism did not seem to offer an advantage per se, but a higher flow at low oxygen levels seemed to be an advantage. The preload required to produce the maximum output varied with both the delivery method *and* the oxygen level (Figures 6F and 6G).

6.5 DISCUSSION

Oxygen inhalation therapy has long been used in the treatment of patients with acute myocardial ischaemia, particularly when ischaemia is complicated by hypoxaemia. It

seemed logical therefore, that increasing oxygen delivery during the induced ischaemia required for open heart surgery, would be beneficial.

This has resulted in a recent trend during routine open heart surgery to provide oxygen with cardioplegic perfusates, initially by use of cold blood based solutions[220], subsequently by bubble or membrane oxygenation of crystalloid solutions[222], and now more recently, by continuous warm blood cardioplegia[223]. There remains a considerable controversy, however, as to the optimal method[229]. The implementation of these techniques acknowledges the fact that whilst cold chemical cardioplegic arrest is associated with much reduced oxygen consumption, there is still a finite requirement which may be detrimental if not met. How much oxygen needs to be provided is still unclear. Coetzee[225] studied the effects of delivering cold intermittent oxygenated (pO_2 440 mmHg) crystalloid cardioplegia in isolated rat hearts compared with the same aerated solution, at both 4^0C and at 20^0C. The hearts receiving the oxygenated solution showed marked superior post storage function and high energy phosphate preservation. Interestingly, the group stored at 20^0C with oxygenated solution, had the best overall results. The studies of Bodenhamer[119] also showed overwhelming improvement with oxygenated crystalloid cardioplegia in an ischaemic dog model.

Guyton[214] performed studies on dogs with the same qualitative results and went on to carry out prospective randomised clinical studies on patients undergoing routine coronary artery surgery. These latter studies showed marked differences in the isoenzyme CK-MB release between the groups, at aortic clamp times in excess of 28 minutes. These findings, along with similar literature reports, prompted investigations into the use of blood as a cardioplegic vehicle.

A number of studies, with conflicting results, have been published; Engelman[226] used an *in-vivo* isolated pig model to compare oxygenated and aerated crystalloid solutions, delivered at 4^0C, with an oxygenated blood cardioplegia, delivered at 15^0C. The blood based solution demonstrated superior high energy phosphate preservation, following 3 hours intermittent perfusion. In a study using an isolated in-vivo dog heart preparation Magovern[227] compared aerated crystalloid with blood cardioplegia, with both solutions delivered at 4^0C, 10^0C and 20^0C. The crystalloid group delivered at the two colder temperatures showed equivalent preservation to blood cardioplegia at 20^0C, which was inferior to crystalloid at the colder temperatures. These studies serve to illustrate that whilst blood can carry much more oxygen than crystalloid solutions, at temperatures below 15^0C the leftward shift of the oxyhaemoglobin dissociation curve severely limits oxygen delivery[228].

The above testifies to the limitation of blood as a cardioplegic base for donor heart preservation, although washed erythrocytes may be a useful additive due to their increased buffering capability[229] and their rheological benefits[230].

Recent studies by Wicomb[141], who was able to obtain successful 24 hour preservation of rabbit hearts, suggest that only enough oxygen to support anaerobic metabolism is required and that even lower flows may be sufficient. However, the inclusion of an effective colloid seems to be essential for this approach to be effective. *Microperfusion*, as described by this group, involved perfusion rates of only 0.25 $ml.g^{-1}.24\ hrs^{-1}$ of non oxygenated perfusate (modified University of Wisconsin)[23], compared with 48 $ml.g^{-1}.24\ hrs^{-1}$ in the **CP** group in the above studies and perfusion flows of over 1.5 $l.g^{-1}.24\ hrs^{-1}$ in conventional perfusion preservation techniques. However, Wicomb found that a high molecular weight polyethylene glycol (PEG20) was the only colloid which allowed

these impressive results to be achieved, and that the storage temperature needs to be close to zero. The group ascribe the success of the technique to the ability of PEG to ensure uniform perfusion, the provision of substrate and enough oxygen to support glycolysis and a flow rate just high enough to prevent acidosis and ensure the removal of waste products. However, PEG20 turns out to be an unstable solution, giving rise to doubts over the reliability of this technique.

Despite the increased interest in the application of oxygenated solutions for routine cardiac surgery only 5% of Centers surveyed reported using oxygenated solutions for clinical *donor* heart preservation (Appendix A). The renewed interest in continuous perfusion has likewise not been reflected in donor preservation practice, for the same reason. The differences between cardioplegic delivery in routine cardiac surgery and in the setting of donor heart retrieval, have been discussed in Chapter 5. However, even for those Centres which believe in the efficacy of delivering oxygen during global ischaemia, and in perfusion preservation, the implementation of these techniques is limited by the logistical constraints of providing a suitable methodology.

The choice of a storage time of three hours, used in the studies described in this chapter, was based on previous experience with this model (Chapter 5) which has shown that better than 95% of hearts stored, using the worst preservation method, perform measurable post storage work, after storage for 3 hours. This study produced post storage functional differences of between 49% and 81% of controls, so achieving adequate sensitivity (Figure 6I).

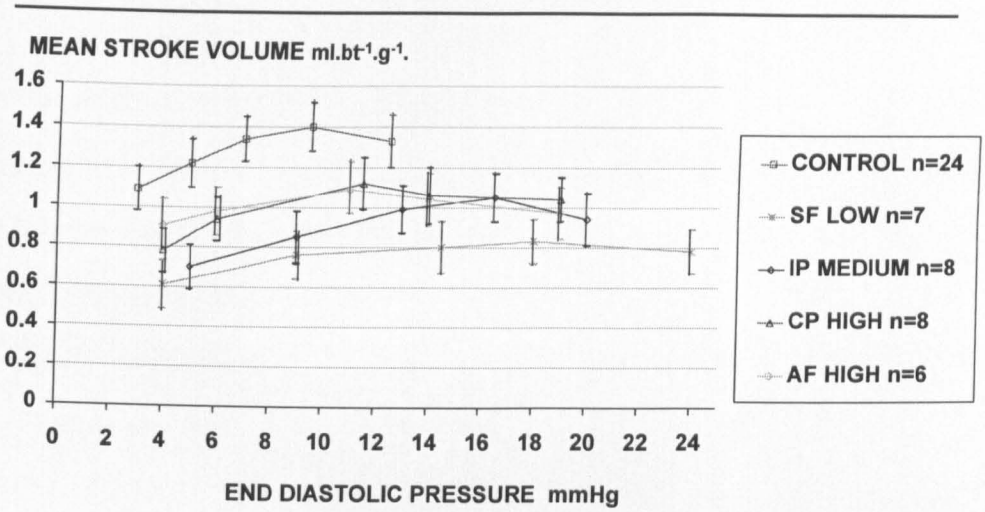


Figure 6I In this illustration the current clinical technique of single flush low oxygen (SF low) is compared with the oxygen level giving the best results in each of the other delivery methods. The pooled pre-storage curve is displayed for comparison. It is clear that all of the alternative techniques offer the prospect of improved systolic and diastolic functional preservation.

The studies described in this chapter underscore the fact that the provision of oxygen to globally ischaemic hearts is generally beneficial, and this finding is increasingly confirmed in the literature[214, 231], with the exception of Wicomb. Figure 6I illustrates the differences between the current technique of **SF** with **LOW** oxygen, as used at Papworth and most other transplant centres (Appendix A) and the improvement possible with low flow oxygenated delivery of the same solution, **CP HIGH**. It also demonstrates that oxygen delivery, under these circumstances, is a much more complex issue than merely meeting oxygen demand, and that the method of delivery is clearly interactive with oxygen *tension*, and possibly other characteristics of the solution. This finding is also reported in a recent study by Cason[232] who used a pig

model in which the left anterior descending coronary artery was selectively cannulated, thus allowing flow and oxygen content to be studied separately.

The application of perfusion techniques to donor heart preservation for clinical transplantation has involved the use of heart-lung auto-perfusion systems[233], portable hypothermia machines[140] and more recently, continuous machine blood perfusion at 34°C[137]. All these techniques are relatively complex and expensive, thus limiting their application. Low-flow perfusion has been applied by others to organ preservation[210,234-236] with some promise.

6.6 CONCLUSIONS

This Chapter focuses on the important interactions between oxygenation and method of delivery. The results clearly show that enhanced oxygen delivery can be beneficial, but that delivery method is equally important, possibly due to the effects of removing metabolites from the vascular system. Solutions with a high oxygen content offer an approximate 20% improvement in function, when delivered by continuous perfusion, compared with unoxygenated single flush delivery. However, the combination of high oxygen content with non-continuous delivery seems to be detrimental. Intermediate levels of oxygenation give improved post storage function with all methods of delivery.

In the following chapter the effects of combining this delivery technique, with improved solutions, are explored.

CHAPTER 7

FUNCTIONAL STUDIES ON FOUR SOLUTIONS

7.1 INTRODUCTION

Extending the preservation time limits of the donor heart has become an elusive goal to researchers in cardiac transplantation. The extent to which donor factors rather than pure preservation factors, are involved, has been unclear, as discussed in earlier chapters. The introduction of strikingly different solutions, such as University of Wisconsin (UW) has enabled this goal to be realised for intra-abdominal organs[31,32] but the results for thoracic organs have been disappointing[237]. In the previous chapter the author was able to show that the delivery of oxygen to globally ischaemic guinea pig hearts, has the potential to improve post storage function by about 20%, using a basic hyperkalaemic cardioplegic solution, as a vehicle. However, there is now an impressive literature[23,24, 237-240] which shows that this type of solution, whilst perfectly satisfactory for uncomplicated routine cardiac surgery, offers sub-optimal protection for donor hearts. This is reflected in the universally accepted cold storage limit of approximately 4 hours for human donor hearts[37]. The differences in the prevailing conditions during open heart surgery compared with donor heart storage, have been discussed in earlier chapters. The effects of varying numerous constituents and conditions have been widely explored in the laboratory such as the influence of extracellular compared with intracellular composition[240], the optimal osmolarity[26] and pH[241,242], the use of different buffers[27], the addition of substrates[24,243,244], the use of various impermeants[245,246], the use of free radical scavengers[247], the addition of calcium antagonists[248,249], negative inotropic agents[250], the addition of flavines[251], and local anaesthetic agents[252] . Whilst

many of the laboratory studies have been encouraging, a method for significantly improved clinical donor heart preservation has yet to emerge[240].

Our own clinical QBM data showed significant impairment in approximately 40% of donor hearts[82], the ISHLT Registry[19], shows 26% of deaths to be graft related. In this chapter four representative solutions (intracellular, extracellular, Histidine buffered and low potassium) used for studies of induction in Chapter 5 have been used in combination with the best technique (continuous low-flow perfusion with a high oxygen content **CP HIGH**), identified in Chapter 6, so as to explore the effects of increased oxygen delivery with *different* solutions. The author wished to determine whether the benefits of increased oxygen delivery, demonstrated with a simple extracellular solution would be common to other representative solutions. Since the author expected an overall improvement in function, the preservation time was extended to 4 hours in order to increase the sensitivity of the model.

7.2 METHOD

Twenty isolated guinea-pig hearts were studied before and after storage in one of four cardioplegic solutions (n=6 per group); Bretschneider HTP, Plegisol, Wicomb and St.Thomas' No.1, for 4 hours at 4°C. Hearts were arrested with 15ml/kg of the cardioplegic solution under test, and subsequently low-flow perfusion stored with the oxygenated solution. The composition of these solutions and the methods of organ removal, functional evaluation and cardioplegic induction were as described in Chapter 5, with each heart acting as it's own control.

Each solution was delivered by continuous perfusion at 4 ml/hr and with a pO_2 of 950 mmHg (**CP HIGH**), as described in Chapter 6.

Function curves were generated before and after storage using the procedure described in Chapter 4.

The groups were compared with respect to :

1. Mean Stroke Volume (LAP 3.7 to 18.5 mm Hg)
2. Maximum Stroke Volume
3. Mean Stroke Work (LAP 3.7 to 18.5 mm Hg)
4. Maximum Stroke Work
5. LAP_{max}
6. Myocardial oedema (wet:dry weight)
7. Heart Rate

Analysis

For each animal a pre and post-storage Function Curve for Stroke Volume and Stroke Work was conducted. For each of the above parameters the post-storage Function Curves were analysed separately by analysis of variance (ANOVA), taking into account the type of solution used. The respective parameter from the pre-storage Function Curve was used as a covariate.

If a parameter was found to have a statistically significant solution effect, then solutions were compared pairwise, using Fisher's LSD method.

Heart rate was analysed in this series, as there was an apparent solution effect.

7.3 RESULTS

Mean Stroke Volume

There was a statistically significant difference between mean levels of Stroke Volume for the four different solutions (p=0.002). The solutions were ranked as follows:

Bretschneider HTP < St.Thomas' No.1 < Plegisol < Wicomb

In the above and in the following comparisons, solutions underlined by the same line are *NOT* statistically significantly different (p> 0.05).

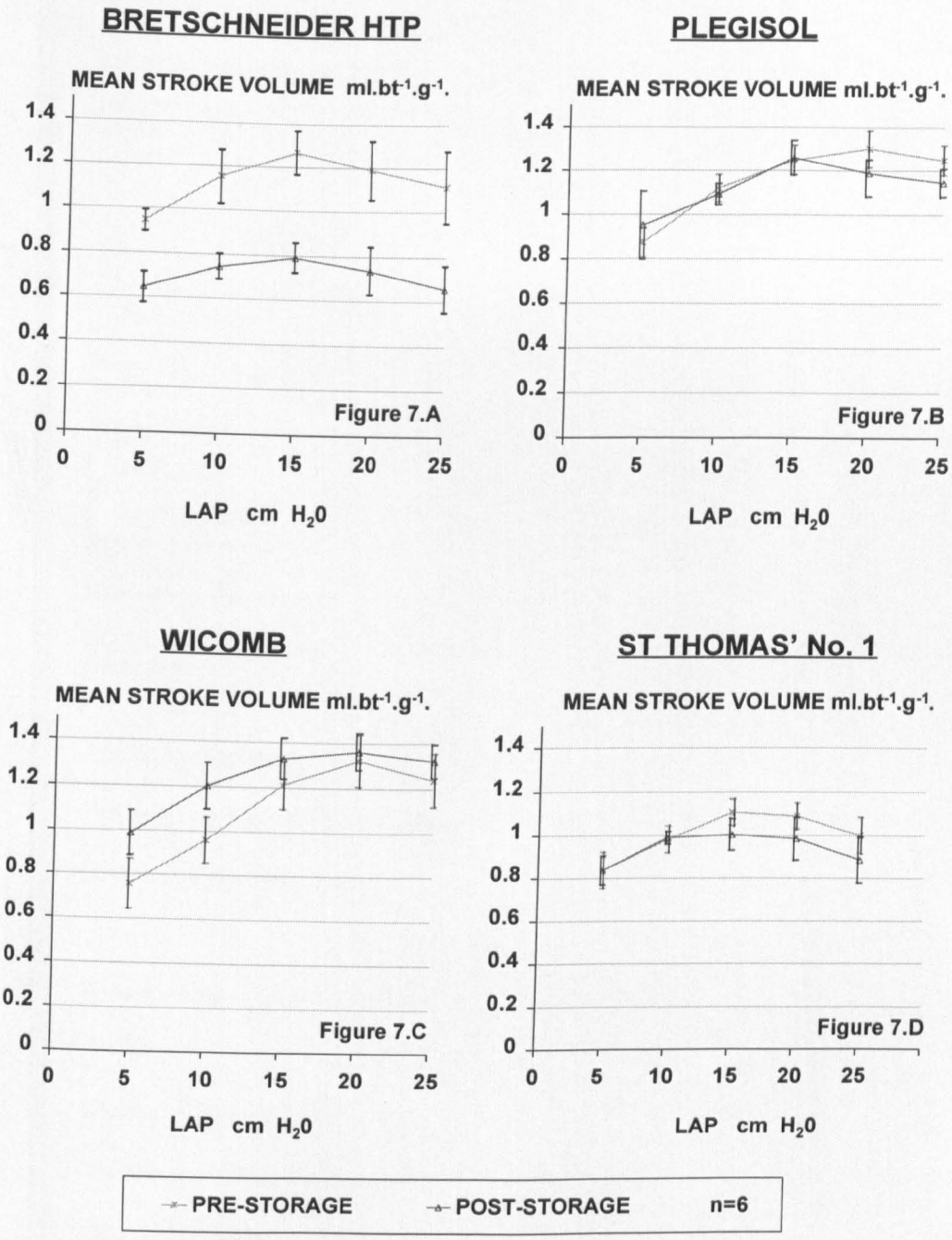
A number of the hearts stored in Plegisol or Wicomb, had mean Stroke Volumes greater than pre-storage levels (Table 7.1).

Maximum Stroke Volume

The results for maximum Stroke Volume are very similar to those of the mean. There were significant differences between the solutions (p=0.002) with the same ranking.

Pre and post storage Stroke Volume Function curves are illustrated in Figures 7A to 7D.

PRESERVATION OF FUNCTION



Figures 7A-7D The above function curves illustrate the changes in stroke volume in response to incremental loading for the four solutions following storage for 4 hours. The pre-storage curve is presented for comparison in each case. Note that in Wicomb solution post storage function was *better* than in the controls values.(1cm H_2O = 0.74 mmHg)

LAP_{max}

There was no statistically significant difference between the preloads at which Maximum Stroke Volume was attained for the four different solutions (p=0.648). Mean loading pressures for the four solutions ranged between 11 and 13.3 mm Hg (Table 7.1).

TABLE 7.1

POST STORAGE STROKE VOLUME $ml.g^{-1}.m^{-1}$.

SOLUTION	MEAN	MAXIMUM	LAP_{MAX}
BRETSCHNEIDER	0.71	0.82	11.8
PLEGISOL	1.12	1.26	11.8
WICOMB	1.24	1.41	13.3
ST. THOMAS	0.95	1.08	11.1
SD	0.21	0.23	2.9

Means and Standard Deviations (n = 6) adjusted for the respective Pre-storage statistic, in each case. LAP is in mmHg.

Mean Stroke Work

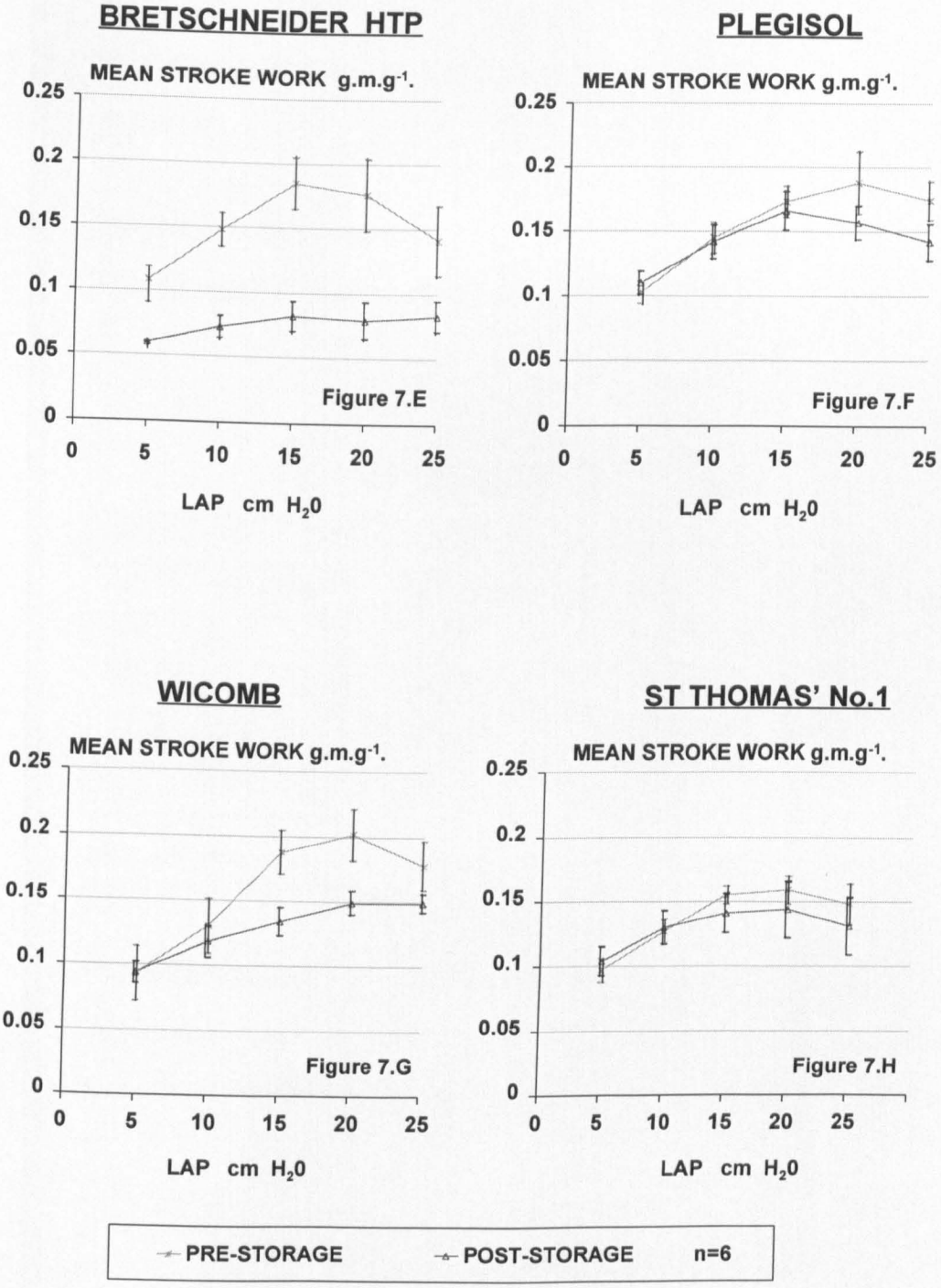
There was a statistically significant difference between the mean levels of Stroke Work for the four different solutions (p=0.008). The solutions were ranked as follows:

Bretschneider HTP < Wicomb < St. Thomas No.1 < Plegisol

Maximum Stroke Work

The results for the maximum Stroke Work were very similar to that of the mean. There were statistically significant differences between the solutions (p=0.011) and the rankings were the same as for the mean (Table 7.2). Pre and post-storage Stroke Work Function curves are illustrated in Figures 7E to 7H.

PRESERVATION OF FUNCTION



Figures 7E-7H The above function curves illustrate the changes in stroke work in response to incremental loading for the four solutions following storage for four hours. The pre-storage curve is presented, for comparison, in each case.(1 cm H_2O = 0.74 mmHg)

LAP_{max}

There was a statistically significant difference between the preloads at which maximum Stroke Work for the four different solutions was attained (p=0.052). Maximums in Wicomb perfused solutions required higher preloads than the other three solutions (Table 7.2).

The solutions are ranked as follows:

Bretschneider HTP < St. Thomas No.1 < Plegisol < Wicomb

TABLE 7.2

POST STORAGE STROKE WORK $g.m.g^{-1}$.

SOLUTION	MEAN	MAXIMUM	LAP_{MAX}
BRETSCHNEIDER	0.0715	0.0863	11.1
PLEGISOL	0.1419	0.1657	12.6
WICOMB	0.1271	0.1533	15.5
ST. THOMAS	0.1330	0.1576	12.6
SD	0.0337	0.0405	2.4

Means and Standard Deviations (n = 6) adjusted from the respective Pre-storage statistic, in each case. LAP is in mmHg

Myocardial Oedema

There were no statistically significant differences between the wet:dry weight ratios for the four solutions (p=0.738). Mean weight ratios ranged from 7.88 to 8.10 (Figure 7I).

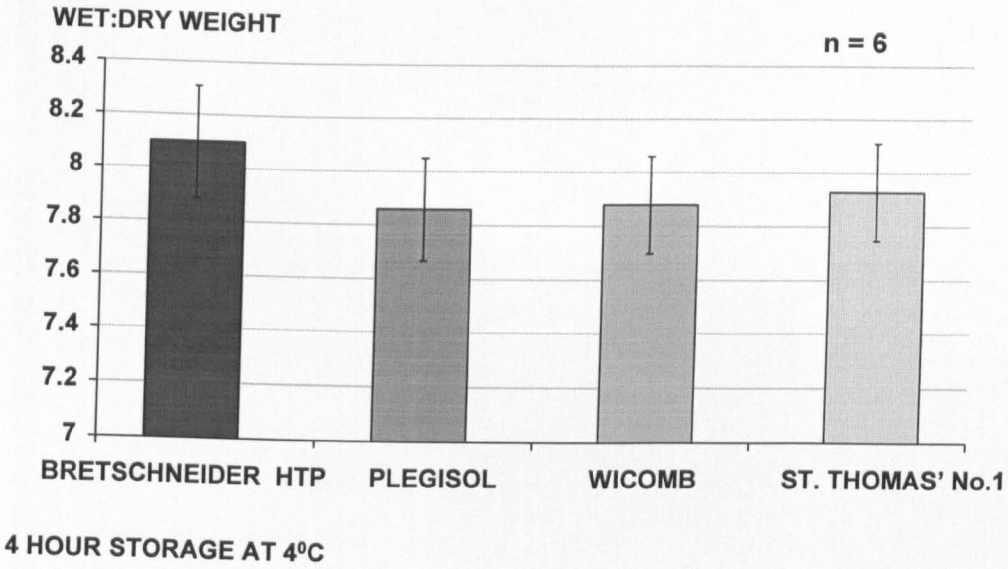


Figure 7I The wet-dry weight ratios, as an index of post storage oedema, showed no difference between the solutions. Bars indicate SEM.

Heart Rate

The mean heart rate was much lower in hearts perfused with Wicomb solution than the other three cardioplegic solutions. This was reflected in the test for solution effect in the analysis of variance, where the p value was small (p=0.052) but not quite statistically significant.

However, the same possible outlier in the Bretschneider group, as identified in Chapter 6, had a post storage heart rate of 160 bts/min. If this value is excluded from the analysis, the mean heart rate for the Bretschneider group is increased and the variability of the data reduced, by 44%. This reduces the p value to p=0.001 (Table 7.3).

The means for the four solutions are ranked as:

Wicomb < Plegisol < St.Thomas No. 1< Bretschneider HTP

TABLE 7.3

POST STORAGE MEAN HEART RATES

SOLUTION	MEAN	ADJUSTED MEAN
BRETSCHNEIDER HTP	247	263
PLEGISOL	240	239
WICOMB	200	200
ST. THOMAS No.1	241	240
SD	29.7	22.2

The adjusted mean takes into account the exclusion of a single outlier in the Bretschneider Group.

Summary

The four solutions had differing effects on the functional preservation of guinea pig hearts, stored using continuous low-flow perfusion with oxygenated solution, for 4 hours at 4^0C.

Bretschneider HTP produced the worst overall preservation of function, in all respects. Plegisol and Wicomb produced the best overall results most clearly demonstrated in Figure 7J in which End Diastolic Pressure is plotted against Stroke Work.

Storage in either Wicomb or Plegisol resulted in post storage performance equal to pre storage values (Table 7.4).

TABLE 7.4

MEAN STROKE WORK VS LEFT ATRIAL PRESSURE

LAP mmHg ($cm H_2O$)	PERFUSION SOLUTIONS				
	CONTROL	BRET HTP	PLEGISOL	WICOMB	ST THOM NO. 1
3.7 (5.0)	0.102	0.060	0.110	0.093	0.081
SEM	(0.028)	(0.029)	(0.026)	(0.023)	(0.035)
7.4 (10.0)	0.137	0.073	0.141	0.118	0.130
SEM	(0.032)	(0.025)	(0.036)	(0.033)	(0.027)
11.1 (15.0)	0.175	0.086	0.166	0.153	0.157
SEM	(0.036)	(0.034)	(0.040)	(0.027)	(0.024)
14.8 (20.0)	0.181	0.079	0.157	0.149	0.145
SEM	(0.037)	(0.043)	(0.036)	(0.026)	(0.025)
18.5 (25.0)	0.161	0.060	0.142	0.148	0.131
SEM	(0.039)	(0.040)	(0.039)	(0.019)	(0.032)

MEAN END DIASTOLIC PRESSURE VS LEFT ATRIAL PRESSURE

LAP mmHg ($cm H_2O$)	STORAGE SOLUTIONS				
	CONTROL	BRET HTP	PLEG	WICOMB	ST THOM NO.1
3.7 (5.0)	2.93	3.10	3.31	3.73	4.88
SEM	(0.00)	(0.10)	(0.80)	(0.70)	(1.60)
7.4 (10.0)	5.15	5.25	5.31	5.44	6.38
SEM	(0.70)	(0.80)	(1.20)	(1.10)	(2.10)
11.1 (15.0)	7.23	8.92	7.81	8.44	8.91
SEM	(1.20)	(1.10)	(1.20)	(1.80)	(2.20)
14.8 (20.0)	9.65	10.94	9.25	11.43	12.12
SEM	(1.40)	(1.60)	(2.30)	(1.20)	(2.10)
18.5 (25.0)	12.4	15.20	11.72	13.63	15.32
SEM	(2.80)	(2.10)	(2.10)	(2.90)	(3.30)

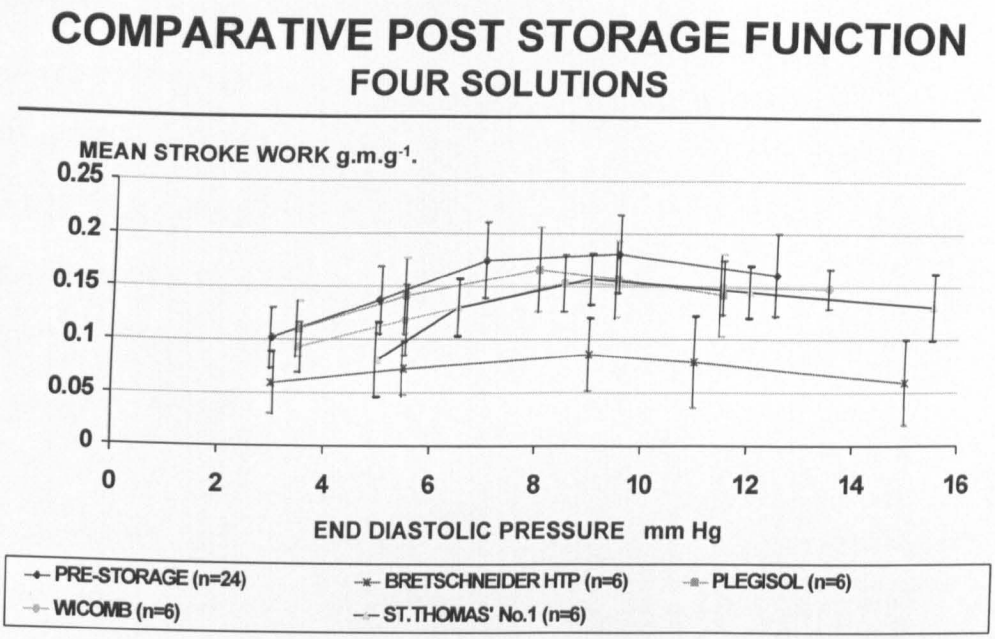


Figure 7J In the above figure all four solutions are compared with their group control (pre-storage), following storage for four hours. It should be noted that not only is the maximum stroke work decreased in St Thomas No.1 and Bretschneider HTP solutions, but that the function curve is distorted at higher preloads - indicating a loss of diastolic compliance. For SEM please refer to Table 7.4.

The differences in ranking between maximum Stroke Volume and maximum Stroke Work can be explained by the difference in heart rates, between the groups. A lower heart rate produces the effect of increasing the Stroke Volume and reducing the Stroke Work, due to a reduction in cardiac output.

7.4 DISCUSSION

This chapter has explored the effects of combining the best method of oxygen delivery, as described in Chapter 6, with a selection of different cardioplegic solutions. The same working heart model, as previously described, was employed and derived parameters from function curves used, as primary outcome measures. The

combination yielded an improvement in post storage function from 59% of pre-storage maximum Stroke Volume achieved with single flush non oxygenated St. Thomas' No.1 (**SF LOW**) following storage for 3 hours (Chapter 6), compared with 107% of pre-storage maximum Stroke Volume seen with the use of low-flow perfusion of oxygenated Wicomb solution, following 4 hours storage, as shown in this chapter (Figure 7K). In a few tentative additional experiments, this improvement would seem to be maintained beyond 6 hours storage.

It is possible that the optimum oxygen content and mode of delivery, might be different for each of the additional solutions and this would need to be evaluated in order to be sure that the best combination of characteristics was being employed. However, the results do suggest that, independent of the benefits conferred by the formulation of a "bespoke" solution such as Wicomb, oxygenation is associated with superior functional preservation.

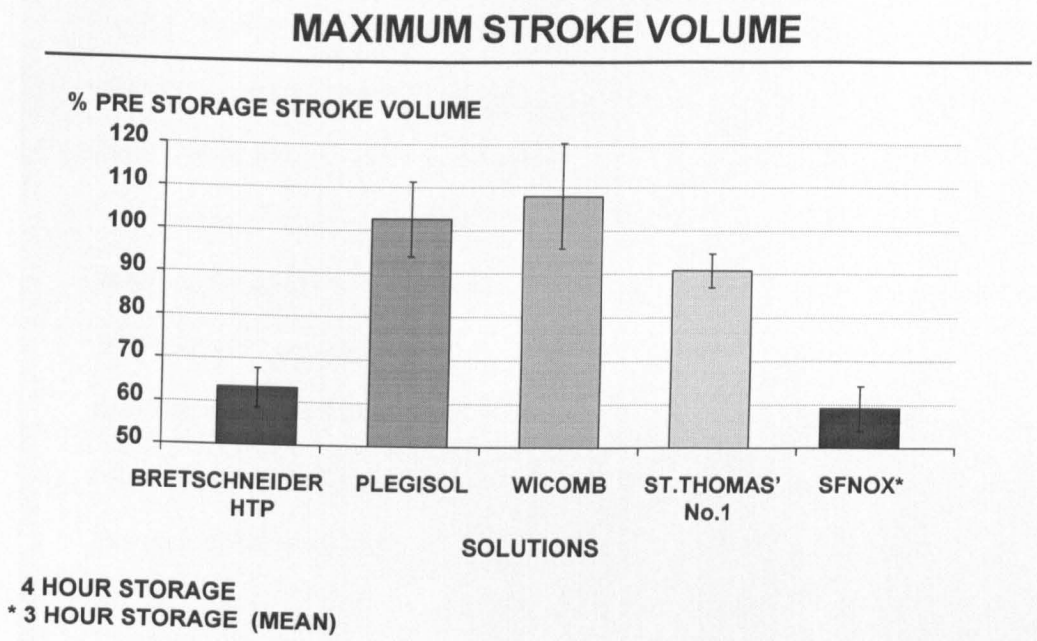


Figure 7K In the above figure the percentage change in stroke volume, with respect to the pre-storage value, is compared for the four solutions and the current clinical method of single flush non-oxygenated St Thomas' No.1 cardioplegia (SFNOX). It is clear that the use of either Plegisol or Wicomb with oxygenation and continuous perfusion offers considerable promise of improvement.

This finding is not altogether surprising since, as discussed in Chapter 5, whilst all of the solutions, other than Wicomb, are commonly used for donor heart preservation, only the Wicomb solution was specifically designed for this purpose (but not yet clinically employed).

Other investigators[23,24, 237-240] have reported on the limited efficacy of the extracellular type formulations, characterised by the St. Thomas' No.1 solution, in models representing donor heart preservation. Gott[240] and associates surveyed US transplant Centres in 1990 and found 42% (51% in the author's survey) to be using extracellular cardioplegic formulations for donor heart storage. They subsequently showed marked

differences in post storage functional performance of isolated blood perfused dog hearts following, 6 hours storage intervals in an extracellular (EC) solution compared with the intracellular (IC) formulations of Stanford and University of Wisconsin (UW). Only 1/14 of the EC group was capable of adequate response to volume loading whilst all of the IC hearts (15/15) performed acceptable post storage work. However, in the author's model the only IC solution (Bretschneider HTP) produced the worst results. Swanson and colleagues[23] also used an isolated canine model to compare UW with Stanford and a modified Collins solution (all IC formulations). There were only minimal differences after 5 hours storage but after 12 hours only the UW solution allowed rapid recovery of function despite similar ATP levels in all three groups.

Recognising that there are a number of distinct steps which may independently account for donor heart damage, Burt and Copeland[253] used an isolated working rabbit heart preparation to look at the impact of the various stages of retrieval and transplantation. Simple cooling and reperfusion caused no loss of function but recovery was slow. Cardioplegic induction prior to cooling and reperfusion recovered 95% of function whilst the same treatment with ischaemia at 25^0C showed 89% recovery. The same regime with the addition of 24 hours storage only recovered 84% of systolic function and also showed diastolic dysfunction. This suggested that whilst induction, ischaemia and reperfusion all play a part in damage to the donor heart, it is the protracted storage time which makes the major contribution. In contrast, Minten[254] and colleagues found that initial cardioplegic induction maintained high energy phosphates at approximately 80% of control levels after 3 hours of hypothermic ischaemia whilst the same storage conditions without cardioplegic arrest resulted in only 20% of initial ATP levels to be maintained. The literature is replete with similar apparent contradictions.

Throughout the literature, however, perfusion preservation has been shown to offer the most effective method of protecting the donor heart[25,132,158,255,256]. This had advanced to the point where clinical application was used by the Cape Town group[140]. However, of the four clinical hearts, preserved for between 7 and 17 hours using hypothermic crystalloid perfusion preservation, only one subsequently recovered normal function, on the third postoperative day. These patients would probably all have died had they not been using the technique of heterotopic cardiac transplantation, in which the inotrope supported patient's own heart kept the patient alive while the donor heart recovered. This experience also served to point out the disparity between their baboon preservation model, in which they had routinely achieved excellent 24 hour perfusion preservation[257], and the clinical situation.

Evidence that the problem of transfering encouraging laboratory findings to clinical therapy, in the case of cardiac donor preservation, can be found in the comparative time intervals between the publication of these laboratory findings and the lack of any clinical reports of successful applications. Reports of the superior performance of the various iterations of UW solutions for cardiac preservation, in the laboratory, appeared as early as 1988[23] but the technique has yet to be used clinically. In contrast to this, laboratory reports of the use of UW solution in liver transplantation also appeared in 1988,[258] followed by the first publication of a successful clinical application, in the same year[259].

7.5 CONCLUSIONS

In this chapter the utility of the isolated working heart model, used in the controlled environment of the laboratory, has been demonstrated. This was used to explore the potential benefits of oxygen delivery, as developed in Chapter 6, used in combination

with improved solutions, to provide better functional preservation of donor hearts. This approach has allowed an approximate doubling of functional preservation, compared with the current clinical technique of flush-store with non-oxygenated St.Thomas' No.1 cardioplegia. However, as is evident from the literature quoted above, successful transfer of these findings to the clinic requires the integrated approach outlined in the early chapters of this thesis.

CHAPTER 8

GENERAL DISCUSSION

8.1 SUMMARY

Heart transplantation has been one of the success stories of twentieth century medicine, from it's inception in the late 1960's to the present day. Surprisingly, however, there has been little progress in clinical donor organ preservation during this 25 year history[8,260-265], despite impressive improvements in Immunosuppression and other aspects of patient management. This is in stark contrast to the progress made in the preservation of other organs, where the safe storage time has been more than quadrupled,[31,32,266].

The studies described in this thesis were prompted by the frustrations which the author encountered as a founding member of the first centre in the UK to establish a heart transplant programme, in 1979. Many of the logistical problems associated with the peri-operative events surrounding the conduct of the implant have their genesis in the constraints imposed by the limited safe time available for donor organ preservation. In addition, the possibilities of improved organ matching are similarly constrained by the limited available time. Subsequent early morbidity and mortality is also strongly influenced by the functional capacity of the transplanted heart[100,262] and this is at a level comparable to that caused by the complications of rejection and infection[20]. More recently evidence has been produced to suggest that early peri-operative ischaemic damage to the coronary endothelium may be related to the generation of accelerated

graft atherosclerosis[266], the most significant cause of late mortality in heart transplant recipients[267-268].

The literature is replete with successful laboratory based solutions to the problem of donor heart preservation[21,27,30,46,98,123,132,137,150,158,171,226,231,237,243,247]. However, in contrast to abdominal organ transplantation, there are no reports of this success being transferred into clinical practice. It is the contention of this thesis that the reasons for this poor record are predominantly threefold; a failure to address the impact of brain death and donor management both on the metabolic state of the donor heart and on the interaction of this with the method of preservation. Secondly, the attempt to extend the principles of preservation used in different settings or for different organs, to heart transplantation, and thirdly, the use of laboratory models in isolation or which inadequately reflect the conditions pertaining to heart transplantation.

The central ethos of this thesis is that a combined approach to the problem of donor heart preservation is required; focused laboratory studies using appropriate models, together with controlled clinical evaluation within a tightly managed and controlled donor population.

8.2 CENTRAL ISSUES

As a prelude to the experimental studies described in this dissertation, the author conducted an International Survey into contemporary clinical donor heart preservation techniques[37]. The survey revealed a surprisingly diverse number of techniques to be in use, reflecting the lack of any one reliable method. None of the techniques reported had been designed to meet the specific needs of the globally ischaemic donor heart. A limitation of the survey was that only meaned centre data was available for analysis

and outcomes could only be crudely assessed by 30 day survival. This study was published in the Journal of Heart and Lung Transplantation[37] and a plea made for donor related data to be included in the Registry database. The author is pleased to report that, as of April 1995, this data is now being routinely collected.

The effects of trauma to the brain on the myocardium have been recognised for more than 40 years[44]. However, whilst there is universal agreement that brain death is associated with catecholamine mediated cardio toxic effects[44,45,58,59,72,91], there is less of a consensus with regard to HRT as part of the management regime. Various groups[68-77] have verified the fact that, following brain death, serum T_3 levels are often abnormal. However, some investigators[67,71] have contended that there is no basis for HRT in organ donors. Closer scrutiny, however, shows the latter conclusions to have been made on the basis of inadequate studies. Several other investigators[47,49,50,52,64-67], including the author[43,73,107,112,269], have been able to show that the problem of neurologically mediated cardiac dysfunction in the brain dead donor can be resolved by appropriate management, in most cases, including HRT.

Chapter 3 provides a review of the literature regarding the impact of brain death on myocardial metabolism and function. It was clear that it would be necessary to quantitate and, if possible, manage this phenomenon if a significant impact on safe preservation was to be made. A secondary, but equally important reason, is that the effects of brain death produce a large variability in pre-excision cardiac function, making the objective assessment of any potentially improved technique impossible to determine given the small numbers available for investigation. A series of pilot studies was therefore undertaken, resulting in the establishment of an improved donor

management regime[43], and the development of a Nomogram approach to functional management and assessment[269].

The impact of this regime was subsequently investigated in a study of 150 multi-organ donors and shown to result in an improved donor retrieval rate of some 30% together with a reduction in the variance of pre-excision functional haemodynamics by up to 44%. The recipients of these initially poor, *managed* hearts experienced a post transplant recovery at least as good as those receiving donor hearts which had been retrieved without any special management techniques[107]. These studies had taken place in parallel with laboratory studies aimed at establishing appropriate models for screening potentially improved preservation techniques, and are described in the chapters 4 to 7.

Extending the principles used for routine open heart surgery to donor heart preservation, ignores the fact that significant differences exist between these two situations. The induction of cardioplegic arrest takes place under very different circumstances in routine surgery where the patient is supported on cardiopulmonary bypass, which unloads the heart at the same time as initiating cooling, with oxygenated blood having a reduced haematocrit. The donor heart is exposed to global ischaemia of between 3-4 hours in contrast to the 30-90 minutes of ischaemia in routine surgery during which there is also a variable degree of collateral flow to the coronary vascular bed. The donor heart is usually exposed to temperatures in the range of 4° C in contrast to 15 - 25° C in routine surgery and there always exists a variable degree of metabolic dysfunction in the donor heart, as a consequence of brain death.

Applying to heart transplantation the principles of preservation successfully developed for abdominal organs ignores equally important differences between these organs and the heart. Differences which critically influence the adequacy of preservation relate to cold sensitivity of cation transport[270], membrane permeability to various solutes[271], specific metabolic pathways for energy production[33], and the relative paucity of naturally occurring defence mechanisms against oxygen free radicals[34] and to the inflammatory response[35] in human myocardium. Over and above these important differences is the overwhelming necessity for the heart to perform significant levels of work within minutes of revascularisation. This means that for preservation of donor hearts a number of cardiac specific principles need to be addressed. In essence these are; the prevention of cellular oedema, limitation of calcium overload, the reduction of oxidative damage and the provision of substrates for energy production.

Tissue oedema has long been recognised as a complication of cold storage. This occurs because both anaerobiosis and hypothermia inhibit the sarcolemmal sodium/potassium pump, thereby allowing the entry of sodium and chloride ions down their concentration gradients, as well as subsequent cellular swelling as water passively migrates to equalise transmembrane osmotic gradients[33]. A method of preventing this is to include a cardiac specific impermeant, such as Mannitol or a high molecular weight carbohydrate[240]. Assuming that the calculated osmotic force derived from the intracellular proteins and nondiffusible anions is in the range of 100 to 140 mosmols/kg, then a similar concentration of impermeant in the storage solution will prevent this complication.

Calcium overload is a major feature of ischaemia/reperfusion injury which substantially impairs post ischaemic recovery of function by a contracture-related increase in

diastolic stiffness[269]. This has been observed by several investigators[272] and was demonstrated by the author in the studies reported in chapters 6 and 7. The problem can be counteracted by appropriate electrolyte manipulations which include; lowering the calcium concentration in the solution to around 0.25 mmol/l, the avoidance of potassium concentrations in excess of 20 mmol/l[273] (probably the threshold beyond which membrane depolarisation elicits a rise in cytosolic free calcium), the maintenance of a sodium concentration in the extracellular range (to prevent calcium entry via the sodium/calcium exchange) and the addition of magnesium as this ion competes with calcium at both sarcolemmal and intracellular sites[274]. In addition, keeping the pH slightly acidotic inhibits calcium entry via the sodium/calcium and sodium/hydrogen ion exchange mechanism[275]. Finally, the use of calcium channel blockers might also be useful[51].

There is now overwhelming evidence that oxygen-derived free radicals are important mediators of myocardial damage[200,273,276]. This can be most easily demonstrated during post ischaemic reperfusion but may also occur during perfusion. Free radical damage can be expected to occur during heart transplantation in which two superimposed episodes of global ischaemia (one during storage and the other during implantation) occur before being abruptly reperfused with highly oxygenated recipient blood, via the extracorporeal bypass machine. The protective effects of both interventions which interfere with free radical generation[277] or subsequent free radical activity[278], have been shown, experimentally. However, it is important to ensure that *thiol* based agents are used in their reduced states[279].

An additional important feature of a preservation solution is the provision of substrates or precursors which can limit tissue injury by making possible the production or

conservation of high-energy phosphate compounds, during ischaemia[33]. In the experiments reported by the author in chapters 5, 6 and 7, oxygen was supplied to the tissue by the use of high oxygen containing solutions, allowing oxidative metabolism to proceed at a reduced rate. However, adenosine, aspartate or glutamate (the latter is able to enter into transamination reactions yielding ATP under ischaemic conditions) have also been shown to improve ischaemic tolerance during global ischaemia. In the normal perfused and functioning heart there is a pool of adenine nucleotides of 5-7 μmol/g wet tissue, with ATP being by far the largest fraction[280]. The tissue concentration of total creatinine is two to four times higher than that of the adenine nucleotides and 35% to 50% of this is phophorylated to creatine phosphate. In laboratory animals the CrP:ATP exceeds the value of 1 whilst the human myocardium has smaller relative concentrations of CrP than that of animals (ratio<0.9) but is rich in glycogen. However, the tissue concentrations of many substrates, metabolites and enzymes vary in different regions of the heart, there are species differences and differences relating to age, diet and stress[281]. Steady state tissue concentrations reflect an equilibrium between synthesis and supply, on the one hand, and demand and utilisation on the other. There is a high degree of coupling and therefore any significant disturbance on either side of the equation will render steady states impossible. The interpretation of the significance of any level of tissue substrate therefore has to be cautious[282]. This supports the author's view that, whilst biochemical markers may give useful insights into mechanisms, the best way to measure myocardial functional performance is to measure function.

The addition of oxygen to cardioplegic solutions has gained popularity in the last few years borne out of the recognition that whilst hyperkalaemic cardioplegia can bring about an approximate ten to twenty fold decrease in oxygen demand, there is still a

finite requirement of some 0.5 $ml.min^{-1}.100g^{-1}$ at $4^{o}C$[215]. The mode of delivery has been somewhat controversial in the setting of routine cardiac surgery with cold blood initially being the favoured vehicle[220], but more recently either crystalloid[222] or warm blood vehicles[223] have predominated. The emphasis was initially on the superior oxygen transport of blood[226] until it was realised that, at temperatures below about $15^{o}C$ the leftward shift of the oxyhaemoglobin dissociation curve[228] severely limits oxygen delivery. However, washed erythrocytes may prove to be a useful additive due to their increased buffering capacity[229] and their superior rheological properties[230], and this was the base chosen by the author for the human working heart model.

A number of investigators[23,27] have recently recognised that the above characteristics are necessary features of solutions suitable for donor heart preservation and that the use of either solutions designed for short-term cardiac surgical applications, or solutions designed for the preservation of abdominal organs, are unlikely to yield satisfactory results. Accordingly, a multi-centre clinical trial of at least one of these *bespoke* solutions has been underway since April 1994 but the results have yet to be presented.

Working heart preparations have been used by physiologists and pharmacologists to unravel functional and metabolic mechanisms, for the past 90 years. However, unlike other forms of transplantation, the donor heart is required to perform significant work within minutes of being transplanted. A relevant model therefore needs to evaluate myocardial functional reserve. In Chapter 4 the development of a small working heart model is described in which volume loading is used to stress the heart. Subsequently a human working heart model was established, for pre-clinical evaluation of promising techniques.

This small working heart model was used in the subsequent three chapters to investigate the comparative efficacy of four representative solutions in establishing cardioplegic induction, the interaction of different levels of oxygenation and delivery modes of a storage solution, and lastly the impact of combining the best of these with an improved solution. Oxygen delivery was chosen as a representative method of improving post storage function, from the wide range of potentially effective agents, and this was then comprehensively investigated. A novel method for oxygenating crystalloid preservation solutions was developed[219] to facilitate these studies and to provide a simple yet reliable and practical means of incorporating oxygenation of these storage solutions into clinical practice.

This series of studies demonstrated the potential for more than doubling the current safe preservation time for donor hearts and served to illustrate the utility of this approach.

8.3 IMPLICATIONS

This thesis has focused on a strategy, rather than attempting to provide a solution to the problem of donor heart preservation. The author has established that the supply of donor organs is the most significant factor limiting the expansion of what is currently the only effective therapy for end-stage heart disease. Evidence has been presented to show that the problem of improved donor heart preservation, and retrieval rates, has been resistant to solutions shown to be effective for other transplantable organs. The author has further demonstrated that the use of a donor management regime has both improved the donor retrieval rate and the function of donor hearts. Parallel laboratory studies demonstrated that the use of a small animal working heart model, used as a screening device for potentially improved preservation methods, together with a human

working heart model for pre-clinical screening, offers a potential solution to this dilemma. This work suggests that only by a combined approach is a significant impact on the problem likely to be forthcoming.

8.4 FURTHER INVESTIGATIONS

There are a number of promising avenues to which the principles outlined above could be applied. The issue of controlled reperfusion[283] was not addressed in the models but this could be accomplished by interposing cross-perfusion, with a donor animal, or in the case of the human heart, with fresh human blood, to study this phenomenon. The hearts could subsequently be mounted on the model, for functional evaluation. Specific free radical scavengers[284] could be used to further analyse the interaction of oxygenation and delivery method, shown in Chapter 6. and the issue of the relative importance of perfusion as distinct from oxygen delivery, determined.

It is likely that both the constitution and the temperature of the induction and the storage solutions need to be different[285] since it is known that hyperkalaemic cardioplegic solutions damage the endothelium[286,287] and are toxic[113,] with extended contact times. The use of hyperpolarising[288] rather than depolarising induction solutions are more energy efficient, and should be investigated. The optimum storage temperature[289] is probably higher than the default temperature of 4°C, which is almost universally used, and it would therefore be helpful to conduct a series of experiments to bracket the optimum temperatures for induction and storage. The implications of this are that some form of active temperature control would be necessary. A suitable device has been developed and used clinically by the author, for the past 15 years [279].

This work, and the work of others[158,161,290,291], suggests that a perfusion preservation method would be superior to cold storage but that a simple, safe methodology would need to be developed before clinical adoption would be likely.

It is also clear that solutions with an intracellular composition are superior to those with an extracellular composition[240] and that the use of a large molecular weight impermeant is also effective[246]. It would therefore be worthwhile to reconsider an optimum solution, specifically designed for donor heart preservation, rather than tinkering with solutions which were designed for other purposes.

Recently the phenomenon of *preconditioning* has attracted widespread attention although the finding that short periods of ischaemia, prior to a longer ischaemic episode, confers protection to the myocardium, has been known since the late 1980's[292].

Several groups[30,292-296] are currently investigating the mechanisms in humans, with a view to finding a clinical method for exploiting this effect. Evidence comes mostly from the short ischaemic periods produced during coronary balloon inflation during coronary angioplasty, but this is difficult to extrapolate to global ischaemia. Other human studies have retrospectively examined whether a period of angina, 24 to 48 hours before an acute myocardial infarct confers a protective effect[297]. Recent work suggests that the mechanism involves endogenous ligands such as adenosine, initiating an intracellular pathway by acting on G protein-linked receptors which leads to the activation of protein kinase C and the ATP-dependent potassium channel. The opening of these channels is thought to lead to an influx of potassium into the cell, which shortens the action potential, limits ATP depletion and reduces calcium influx[298]. This mechanism has

been pharmacologically exploited using the agent *aprikalim* to produce a hyperpolarised cardioplegia[288,299] in animal models, to good effect.

There are also a number of other promising pharmacological agents which may have some utility in protection from the effects of global ischaemia, in particular the analogues of adenosine[300,301] which may be acting through the mechanism outlined above. The use of nonvasoactive concentrations of a nitric oxide synthetase inhibitor, which stimulates glycolysis from exogenous glucose, has shown promise in an animal model of ischaemia[302]. Platelet-activating factor antagonists[303] have also been shown to be effective in blocking the platelet mediated inflammatory reactions induced by ischaemia-reperfusion, by competing for PAF receptor sites. The flavonoids, a group of substances which can interact with the cytochrome P-450 system, have recently been shown to be useful adjuncts to cadioplegic solutions used for global ischaemia[251]. The effects on the cytochrome system are thought to be related to promoting increased enzyme-substrate affinity and improvements to the electron transport mechanism. However, flavones are also known to act as antioxidants[304] and antiinflamatory agents[305].

Apart from the increasing use of NMR[190] as a method of evaluating the energy status of tissues a number of new methods pertaining to global ischaemia are now becoming available. Whilst studies of ischaemia/reperfusion have concentrated on myocyte injury, damage to the microvasculature is equally important. It is now recognised that there is a spectrum of microvascular injury ranging from *no reflow* in areas of myocyte necrosis to more subtle functional changes which result in sub-optimal perfusion[289]. The technique of nuclear track emulsion[306] makes it possible to study microvascular competence and hence unravel the causal relationship between perfusion and

myocyte viability. The toxic effects of cardioplegic solutions on the endothelium[288,307] have also long been recognised. Whilst this is not a problem during routine surgery, since collateral flow removes the solutions within 20 minutes, in the setting of cardiac transplantation where contact times are typically between 3-4 hours, this issue assumes a new importance. This lends further emphasis to the need for different inductive and storage solutions for donor heart preservation. Optical methods for tracking peri-operative ischaemia may also be useful such as the recently developed technique of laser-induced fluorescence[308], which is both sensitive and specific.

During the past two years the UK has been divided into 9 regional zones, centred on transplant units and based on population density, for the purposes of donor heart retrieval. In the first year of this arrangement some 300 hearts were retrieved[309] in the UK. Following publication of the results of the donor management regime[43] described in Chapter 3, there has been increasing national (and international) acceptance of this approach. This means that we are now uniquely positioned to carry out meaningful clinical donor preservation studies on a nationally collaborative basis.

The strategy which the author proposes thus consists of; using the small working heart model to screen the most promising techniques, solutions and pharmaceutical agents and selecting the best of these for further study on the perfused human working heart model. Experimental human hearts could be obtained from multi-organ donors who either fail to meet transplant age criteria or who demonstrate an unacceptable level of coronary artery disease. Baseline functional data could be obtained, as described in Chapter 3. This provides a means of testing new techniques on human hearts, from brain dead donors, as a prelude to clinical trials. Multi-centre, fully randomised clinical trials could then be undertaken, with all participants using exactly the same

management and assessment protocols. This would ensure adequate numbers of patients in an experimental environment which reflects clinical practice.

8.5 CONCLUSIONS

Further progress in heart transplantation is donor limited. Efforts to improve the supply, and functional quality of donor hearts has met with little success, despite considerable effort, over the past 25 years. This dissertation has provided information on current clinical practice. A regime for improved donor management has been established and shown to be effective. A laboratory based system for screening potentially improved preservation methods has been demonstrated and validated. A strategy for combining these laboratory studies with clinical trials is advanced. It is suggested that adoption of the above approach offers a way forward in what has been a most refractory problem within an area of medicine that has, in other respects, been a spectacular success story.

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Donor heart preservation survey. 1992

Donor Heart Preservation Survey

Dereck Wheeldon, MIBiol,[a]* Linda Sharples, BSc,[b] John Wallwork, FRCS,[a] and Terence English, FRCS, PRCS[a]

A questionnaire requesting information on donor heart preservation technique and outcomes during the first 6 months of 1990 was circulated to heart transplantation centers worldwide. Seventy-nine usable replies representing 1371 clinical transplant operations were received. Twenty-seven percent of the respondents reported using some form of donor pretreatment. Most (90%) used single flush cardioplegic induction with the use of eight different types of cardioplegic solutions, only 5% of which were oxygenated. Six different types of storage media were used, and the coolant was melting ice in 66% of the centers. Storage temperatures between 0° C and 7° C were reported, with 78% of the respondents using 4° C storage. Fifty-five percent of the centers used some form of reperfusion modification. No statistically significant associations were noted between outcome and technique, apart from the use of storage medium in which the use of cardioplegic solution conferred a 2.5 times increase in deaths compared with cold saline. The results of this questionnaire provide evidence for the diversity of techniques currently used for donor heart preservation, reflecting the lack of any one optimal method. J HEART LUNG TRANSPLANT 1992;11:986-93.

In contrast to kidney and liver preservation scant progress has been made in the field of donor heart preservation over the past decade or more. Many elegant laboratory studies have been published over this time, but their impact on clinical practice has been insignificant. This questionnaire was directed towards establishing a database against which future developments can be compared and in an effort to identify methods in current clinical use that may be offering advantages and that might be associated with poor outcomes. Ninety-two replies were received, of which 79, representing 1371 transplant operations, contained information suitable for analysis. The questionnaire requested information on the following areas: donor pretreatment, cardioplegic induction, transport medium, storage temperature and containers, perfusion preservation techniques, reperfusion, and outcome data. A section at the end of the questionnaire asked for comments on current technique. A limitation of the analyzed data is that only the center, and not the individual patient, data were available for analysis. Donor heart availability is the single most limiting factor in heart transplantation today and one that is not amenable to solution by increased financial resource allocation. In addition, the quality of transplanted hearts in general is still suboptimal, a fact that is reflected by the relatively high mortality from primary graft failure and dysfunction.[1] The authors believe that useful data exist from current clinical practice and that the collection and analysis of this data in a suitable and useful format is a matter of some urgency.

PATIENTS AND METHODS

The questionnaire was compiled in June 1989, and the original version was circulated to six internationally representative transplantation centers for comments on content and style. The updated version was then circulated to 237 centers, based on mailing list information received from the International Society for Heart and Lung Transplantation and the European Society for Organ Transplantation. Questionnaires were circulated prospectively for data requested for the period January to June 1990 to alert the centers to the type of data required. A reply slip indicating willingness to participate in the study was circulated at the same time. In March 1990 those centers not replying were contacted again with the same information. We received 23 replies indicating either a lack of a transplantation center or discontinuation of transplantation activity

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Poster presented at the Twelfth Annual Meeting and Scientific Sessions of the International Society for Heart and Lung Transplantation, San Diego, Calif., April 2-4, 1992.
*Supported by The British Heart Foundation.
Accepted for publication March 25, 1992.
Reprint requests: Dereck Wheeldon, Transplant Unit, Papworth Hospital, Cambridge CB3 8RE, UK.
14/1/38853

at that address. Further reminders and telephone calls were made to nonresponding centers in September 1990 and again in October 1990. We finally received 92 completed questionnaires, of which 79 were ultimately usable. The remaining 13 were rejected because of inadequate information. These usable questionnaires represented 1371 transplant operations.

STATISTICAL ANALYSIS

For descriptive purposes results are expressed as numbers and percentages of centers that returned questionnaires. The numbers of transplantations these centers represent are given where appropriate. Continuous measurements are presented as the mean and range of the means reported for each center.

When comparing the effects of different preservation techniques on outcome, the number of deaths within 30 days, as a percentage of the number of transplantations recorded, was modelled using logistic regression and tested with the likelihood ratio test.[2]

Logistic regression produces results in terms of odds ratios (OR). Given a baseline category to compare with a test category, the estimated odds of dying within 30 days if one is within the test category compared with the baseline category are expressed as an OR; so that 1 represents equal chances and more than 1 represents a greater chance of death.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, Ill.) and EGRET (Statistics and Epidemiology Research Corp., Seattle, Wash.) statistical packages were used for analysis.

RESULTS

Donor Pretreatment

Fifteen (19%) responding centers reported using some form of pharmacologic pretreatment of the donor. Of these, eight centers (53%) reported the use of thyroid hormone (T3/T4), and four centers (27%) used insulin. Altogether nine different treatment regimes were reported in use, either singly or in combination (Table I). No significant differences were noted in 30-day mortality associated with any pretreatment regime.

TABLE I Donor pretreatment

Treatment	No. of centers	Percentage
T3/T4	8	55
Insulin	4	27
Methylprednisolone	3	20
Glucose	2	13
Allopurinol	2	13
Antidiuretic hormone	2	13
Vitamin E	2	13
Mannitol	1	6
Largactil	1	6

No clinically significant differences were found with respect to 30-day mortality.
Pretreat: odds ratio, 0.7636; 95% confidence bounds, 0.4919 to 1.185.
Compared to no pretreatment: $p = 0.238$.
The use of the following treatment combinations was reported by one center in each case: (1) T3/T4, methylprednisolone, allopurinol, vitamin E; (2) T3/T4, insulin, antidiuretic hormone; (3) T3/T4, methylprednisolone; (4) T3/T4, allopurinol, vitamin E; (5) methylprednisolone, glucose.

Cardioplegic Induction

Seventy-three of the respondents (92%) used single flush cold cardioplegic induction, and 69 (87%) of these used the technique with the heart in situ. Seven respondents (8.9%) reported using multiple flush techniques, and two (2.5%) reported bench perfusion only. A combination of in situ and bench perfusion was used by eight of the centers (10%).

No significant differences were found in 30-day mortality associated with the method of induction.

Cardioplegic Solutions

Eight types of cardioplegic solutions were used. Four of these were standard solutions used by 47 centers, representing 879 donor hearts (64%). The remainder were grouped according to whether they were 50% dextrose-based, Krebbs/albumin-type solutions, Plasmalyte (Baxter Laboratories, Deerfield, Ill.)/Krebb's solutions, or blood-based (Figure 1). Only three centers (4%) reported using oxygenated solutions. When divided into intravascular and extravascular type solutions, 39 centers, representing 613 transplantations, reported using intravascular solutions with 12 patients (2%) having difficulty to wean and 61 (10%) 30-day deaths, with one center failing to report outcome.

In the extravascular group 40 centers reported on 795 transplantations, with 14 (1.8%) having difficulty with weaning and 71 (9%) 30-day deaths, with 10 centers failing to report outcome. No outcome differences were noted between individual solutions (Table II).

Storage Medium

Use of four major types of storage medium was reported; the most common of which was cold saline

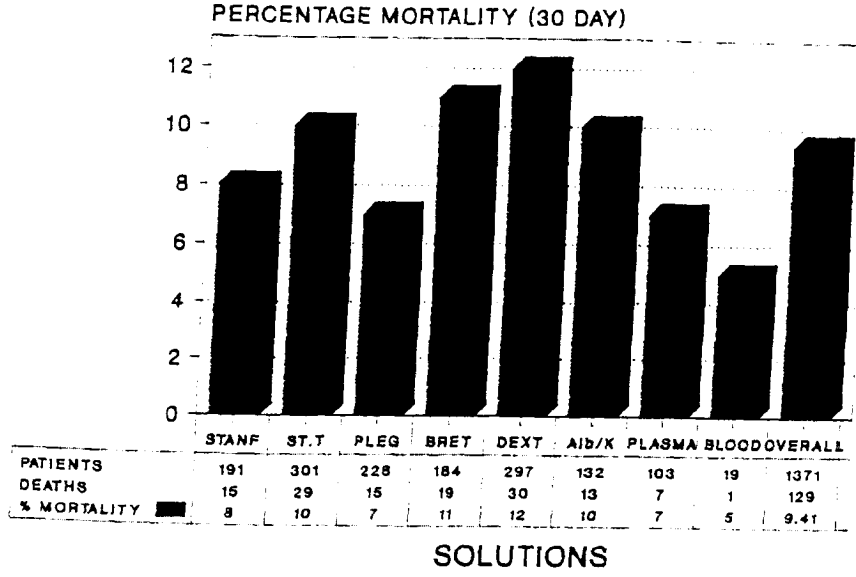

FIGURE 1 Histogram shows the relationship between the percentage of hospital mortality (adjusted for transplantation numbers) and the eight major types of cardioplegic solutions used. *STANF,* Stanford solution; *ST.T,* St Thomas' 1 solution; *PLEG,* Plegisol; *BRET,* Bretschneider HTK; *DEXT,* dextrose-based (excluding Stanford); *Alb/K,* Krebbs-based with albumin; *PLASMA,* Krebbs-based with Plasmalyte; *BLOOD,* blood-based solutions; *Tx,* transplantation.

TABLE II Cardioplegic solutions

	Odds ratio*	95% Confidence bounds
Stanford†	1.570	0.1958–12.59
St Thomas' I†	2.733	0.3514–21.26
Plegisol†	2.043	2.554–16.33
Bretschneider‡	2.124	0.2683–16.82
Dextrose base‡	1.731	0.2194–13.65
Krebbs/albumin‡	2.053	0.2529–16.66
Plasmalyte/Krebbs†	2.864	0.3283–24.98
Compared to blood based	1.000	$p = 0.0685$
Intravascular vs extravascular solutions		
Extracellular solutions	1.410	0.9654–2.060
Compared with intravascular solutions	1.000	$p = 0.074$

*Refers to 30-day mortality.
†Extravascular.
‡Intravascular.

TABLE III Storage solutions

Storage medium	Odds ratio	95% Confidence bounds
Ringer's	1.475	0.7423–2.930
Lactated Ringer's	1.652	0.9721–2.808
Cardioplegia	2.533	1.053–6.093
Other	2.023	1.262–3.245
Compared to saline	1.000	$p = 0.030$

(47%). Nine centers (12%) reported the use of the same cardioplegic solution for storage and induction. Six centers (8%) reported the use of the intracellular Euro-Collins solution for storage. The use of cardioplegic solution as a storage medium was associated with a 2.5 times (OR, 2.53) increase in deaths, compared with cold saline (Table III).

Coolant and Storage Temperature

Most respondents (66%) reported using melting ice as the coolant. Twenty-seven percent reported using iced saline, and a small percentage used an ice/alcohol mixture in combination. Only two centers reported using a commercial storage device. Most centers (73%) reported using 4° C as their storage temperature (Figure 2). Only four centers, however, reported monitoring this temperature, which means that the remainder were actually quoting their own estimates of storage temperature rather than the actual temperature at which the

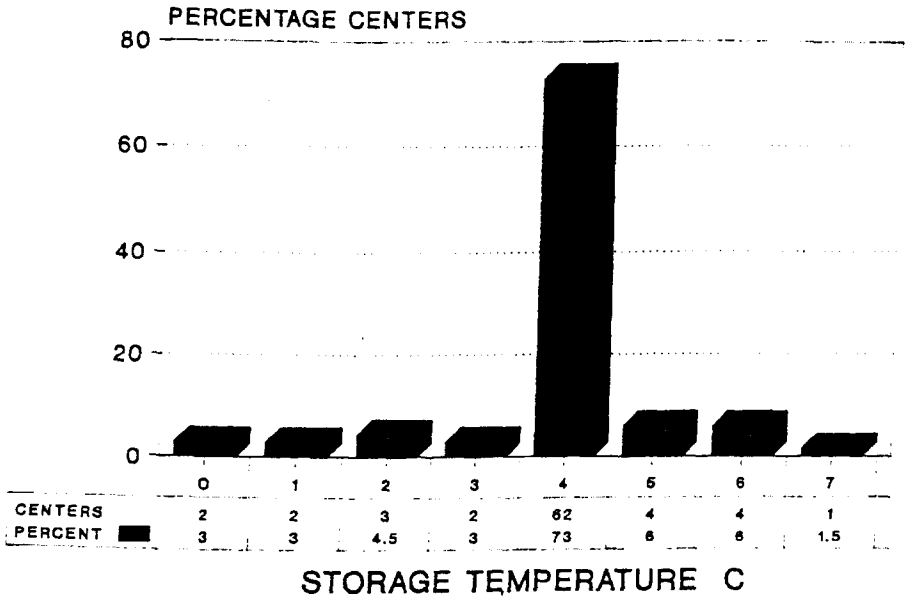

FIGURE 2 Histogram shows the distribution of storage temperatures reported by the responding centers as a percentage of centers.

organs were stored. Only one center reported the use of a temperature-controlled storage system.

No statistically different outcomes were associated with storage temperature.

Perfusion Preservation

One center reported the occasional use of autoperfusion as a method for donor heart retrieval; another reported the use of continuous crystalloid perfusion preservation for hearts retrieved from particularly long distances.

Reperfusion

Thirty-four centers (55% of those responding to this question) reported some type of reperfusion modification, which included secondary blood cardioplegia, pressure control, temperature control, flow control, substrate enhancement not associated with secondary blood cardioplegia, the use of free radical scavengers and osmotic agents, or a combination of these (Table IV). No outcome differences were associated with reperfusion techniques that could be determined from these data.

Outcomes

The mean ischemic time for these data was 151 minutes, with a mean donor age of 28 years. With logistic regression, those centers that stored hearts for more than 3 hours had on average a probability of 30-day mortality that was one half that of hearts stored for less than 2 hours (OR, 0.42). Sixty-four of the centers that responded to this question (85.5%) reported transplanting all of the hearts retrieved, and 71 (95%) reported transplanting at least 90% of the hearts retrieved. Nine hundred seventy-five (87.4%) of the transplant recipients were weaned from bypass without difficulty. One hundred forty-nine recipients (17.4%) required additional inotropes to wean, and a further 230 (26%) required an extended cardiopulmonary bypass to wean. Ninety-three patients (8.7%) failed to be weaned from bypass with these measures, and 52 (5.5%) required intraaortic balloon pumping. A further six patients (0.7%) required a right ventricular assist device; nine (1.0%) required a left ventricular assist device; 17 (1.7%) required biventricular support, and nine (0.9%) required acute retransplantation (Table V).

In the section that asked for responder's comments, 49 (69% of responders) reported that they were completely satisfied with the technique that was currently used in their center. Twenty-two centers (31%) reported some degree of dissatisfaction, however. Of these, 14 centers (64%) expressed a desire for longer "safe" preservation times (12 to 24 hours). Four centers (18%) wanted to see the pretreatment issue resolved. Two centers (9%) desired the efficacy of modified reperfusion to be tested; a further two centers (9%) suggested that a

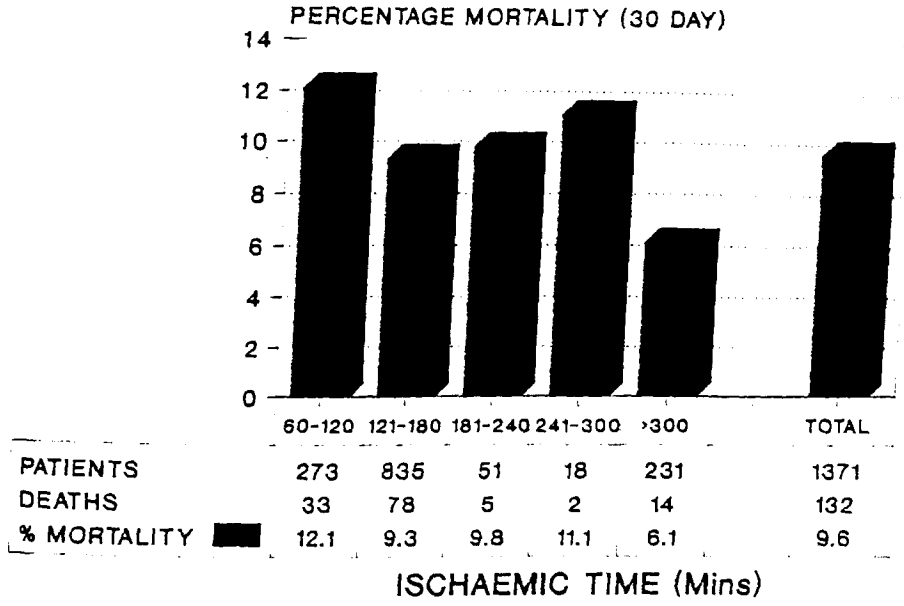

FIGURE 3 Relationship of ischemic times to percentage of hospital mortality, adjusted for transplant numbers.

reliable index of function was required for donor heart evaluation. Another two centers (9%) suggested that a reliable method of temperature control was required. One center (1%) expressed the view that although better preservation methods were probably available, keeping the technique simple was paramount. One center (1%) made a plea for a prospective multi-center randomized trial of the most promising techniques identified by this survey.

DISCUSSION

Donor organ availability is the most limiting factor in the future of thoracic organ transplantation. The importance of organ function after implantation cannot be overemphasized. Early donor heart failure accounts for approximately 26% of the deaths of heart transplant recipients.[2] Evidence exists that some of the problems associated with organ preservation are related to metabolic changes in the donor, consequent on brain death.[3-5] Novitsky et al.[6] have shown benefits from hormone replacement therapy, and recent ongoing studies by our own group show similar benefits. This survey showed no advantage, however, with respect to 30-day mortality, which was the basic outcome measure used throughout the survey.

Careful selection of the donor followed by meticulous management, effective organ preservation, and reperfusion are therefore essential to obtaining optimal postimplantation function. Some degree of physical size matching is also important. None of these is currently well defined, however. Prompted by the shortfall in donor organs, several centers have reported extension of the "classic" donor criteria with respect to age, ischemic time, inotropic support, and donor/recipient size matching,[6-10] without apparent adverse effects on outcome. Liberalization of criteria can, however, produce problems, especially if several contraindications are combined.[11] Outcome is also very much related to recipient characteristics; the International Society for Heart and Lung Transplantation Registry shows that the presence of pulmonary hypertension more than doubles the posttransplant risk of death,[12] and our own risk analysis shows that donor age (increasing) and gender recipient mismatches (female into male) are associated with higher risk.[13]

Many experimental studies have pointed to the superiority of one cardioplegic solution over others, most notably the proponents of the University of Wisconsin (UW) solution[14,15] and Euro-Collins solution,[16] together with the differences between extracellular and intracellular formulations.[17] Clinical evidence of any significant difference is lacking, however, in these and in this survey data, except for an apparent trend of improved results with intra-

TABLE IV Number of centers reporting reperfusion modifications*

Number of centers	Pressure controlled	Flow controlled	Temperature controlled	Enhanced substrate	Secondary blood cardioplegia	Free radical scavengers	Osmotic agents
6	6	–	–	–	–	–	–
2	–	2	–	–	–	–	–
2	–	–	2	–	–	–	–
3	–	–	–	–	3	–	–
2	–	–	–	–	–	–	2
1	1	1	–	–	–	–	–
2	2	–	2	–	–	–	–
1	1	–	–	1	–	–	–
2	2	–	–	–	2	–	–
1	1	–	–	–	–	1	–
2	2	–	2	–	2	–	–
1	1	1	–	–	1	–	–
1	1	1	1	–	1	–	–
1	1	1	–	–	–	–	1
1	1	1	–	–	1	–	1
1	1	–	1	–	1	–	1
1	–	1	1	–	–	–	–
2	–	2	2	–	2	–	–
1	–	1	1	1	1	–	–
1	–	–	1	–	1	–	–
Total no. of centers	20	11	13	2	15	1	5

*Thirty-four centers (55%) are included.
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TABLE IV Number of centers reporting reperfusion modifications*

Number of centers	Pressure controlled	Flow controlled	Temperature controlled	Enhanced substrate	Secondary blood cardioplegia	Free radical scavengers	Osmotic agents
6	6	–	–	–	–	–	–
2	–	2	–	–	–	–	–
2	–	–	2	–	–	–	–
3	–	–	–	–	3	–	–
2	–	–	–	–	–	–	2
1	1	1	–	–	–	–	–
2	2	–	2	–	–	–	–
1	1	–	–	1	–	–	–
2	2	–	–	–	2	–	–
1	1	–	–	–	–	1	–
2	2	–	2	–	2	–	–
1	1	1	–	–	1	–	–
1	1	1	1	–	1	–	–
1	1	1	–	–	–	–	1
1	1	1	–	–	1	–	1
1	1	–	1	–	1	–	1
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B

Multi-organ transplantation: donor management. 1994

Multi-organ transplantation: donor management

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Many studies suggest that only 30% of potential transplant donors will donate organs. Of these, only 70% will become multiple organ donors. On the other hand, up to 40% of patients on transplant waiting lists die before transplantation. Demand clearly outstrips supply. Of the interventions to increase the supply of donor organs, optimal management of the donor patient is receiving increasing interest.

Current Opinion in Anaesthesiology 1994, 7:80–83

Introduction

The majority of potential organ donors have sustained irreversible cerebral injury as a result of trauma or spontaneous intracranial haemorrhage. Whatever the mechanism of injury, as soon as brainstem death has been confirmed, there should be an immediate change in the emphasis of management from that of minimizing the neurological insult to that of maximizing organ function. This is supported by the fact that failure to provide adequate physiological support to potential donors accounts for at least 25% of lost donor organs [1].

The adverse effects of brain death on the heart were demonstrated as early as 1954 [2], and more recent studies in both experimental animals [3,4] and clinical donors [5,6] have suggested that brain death has major histopathological and functional effects. Consequently, without specific intervention, circulatory collapse will usually take place within 72 h of brain death [7]. It is therefore essential that a thorough understanding of the physiological mechanisms involved is attained by those managing the potential donor, if the best use of a scarce resource is to be made. Anaesthetists and intensivists, with their knowledge of cardiovascular and respiratory physiology, are well qualified to make a significant contribution to the care of the donor patient.

This review addresses the main problems which arise in the potential donor and the management strategies to deal with them.

Management of the donor

Cardiovascular dysfunction

Hypotension

This is the most commonly occurring complication in organ donors [8]. At the time of brain death, many donors are already volume-depleted from previous therapeutic interventions used to treat raised intracranial pressure, such as fluid restriction, or use of the osmotic diuretic, mannitol.

Brainstem death itself leads to cardiovascular instability and hypotension of multifactorial origin. Disturbed central regulatory mechanisms cause a loss of vasomotor tone and result in peripheral vasodilatation. Usually, there is an accompanying impairment of myocardial function. This is likely to be of similar aetiology to that of the catecholamine-induced cardiomyopathy of cerebrovascular accidents [6]. Indeed, recently it has been suggested that the more acute and the greater the rise in intracranial pressure prior to brain death, the higher is the catecholamine release, and the more severe is the resulting impairment of myocardial function [9••]. Impaired myocardial performance owing to hypoxia is also relatively common, in our experience, and gives rise to predominantly right heart failure. Deficiencies of plasma hormones may also be implicated in the myocardial dysfunction and hypotension. Novitzky suggested that low tri-iodothyronine levels led to defective aerobic metabolism and a reduction of myocardial energy stores [10]. Low levels of anti-diuretic hormone (ADH) and insulin may result in the development of diabetes insipidus and hyperglycaemia, respectively. The ensuing large urinary losses, and electrolyte imbalances may further compound the pre-existing low arterial pressures.

Comprehensive invasive haemodynamic monitoring, including the use of a pulmonary artery flotation catheter (PAFC), is essential in making objective measurements and optimising management [11]. Preload should be kept as low as possible, consistent with an adequate arterial pressure and cardiac output. The choice of replacement fluid remains controversial, but a regime comprising of 4.3% dextrose/0.25% saline + 20 mM potassium to replace urinary losses, packed cells to maintain a haematocrit level at

Abbreviations

ADH—anti-diuretic hormone; PAFC—pulmonary artery flotation catheter.

© Current Science Ltd ISBN 1-85922-133-5 ISSN 0952-7907

30%, and adjustment of preload with colloid works well [12]. The afterload should be maintained between 800–1200 dynes/s/cm^{-5} with a combination of adrenaline and an ADH (arginine vasopressin). The two drugs when combined are synergistic in their effect and thus only minimal amounts of each are needed to maintain the afterload. In any event, doses of adrenaline and ADH should not exceed 0.5 mg/h and 1–2 U/h, respectively. Hypertension should be treated with sodium nitroprusside. If the minimum haemodynamic criteria cannot be maintained with preloads <12 mmHg, then the inotrope of choice is dopamine because of the loss of sympathetic tone in the brain dead patient. Inotrope dependency at >10 μg/kg/min makes cardiac donation less likely, without more aggressive intervention. The minimum criteria for cardiac transplantation are as follows:

(1) mean arterial pressure should exceed 60 mmHg and,
(2) central venous pressure or pulmonary capillary wedge pressure should not exceed 12 mmHg and,
(3) left ventricular stroke work index should exceed 15 g/m with,
(4) an inotrope dosage of less than 5 μg/kg/min.

Myocardial dysfunction

Patients with cardiac disease are obviously unsuitable for cardiac donation and may require more detailed management including a full clinical history and examination in order to yield other transplantable organs. Intracranial damage leads to frequent abnormal electrocardiographic changes of a pseudo-infarct type [13], the only serious finding being that of *Q* waves. Hypothermia leads to bradycardia and *J* waves which are of no pathological consequence but may require treatment with an isoprenaline infusion or transvenous pacing. Atropine resistance is common [14]. Other dysrhythmias are usually due to electrolyte imbalances. If available, echocardiography may be useful in those cases where serious doubts exist. Paradoxical septal wall motion is a common finding but has no negative prognostic overtones. In older donors, cardiac catheterization and coronary angiography may be justified. Ultimately, most cardiac surgeons believe that the most reliable assessment is by direct inspection of the organ at the time of procurement. However, aborting the procedure at this stage carries considerable logistic and financial penalties. Cardiac arrest is not a contradindication to cardiac donation, provided that resuscitation results in subsequent satisfactory function [15].

Respiratory dysfunction

Pneumonia, aspiration, pneumothoraces, pulmonary oedema and pulmonary collapse commonly occur in potential donors, and may all contribute to a deterioration in respiratory function with subsequent hypoxaemia. The routine respiratory care of the intensive therapy unit, including chest physiotherapy, should continue following brainstem death to avoid these problems. Obtaining an early sputum sample for bacteriological assessment and culture is a useful aid to decision-making with respect to lung donation. Fluid administration is guided by PAFC-filling pressures, and the lungs are ventilated with large tidal volumes (15 ml/kg) to avoid pulmonary atelectasis. Positive end-expiratory pressure may also be used, although levels above 7.5 cmH_2O are best avoided because of the adverse effect on cardiac output and increased risk of pulmonary barotrauma [16•].

Endocrine dysfunction

Impaired hormone release has already been alluded to above. However, the results of hormone replacement therapy reported by Novitzky *et al.* [10] have not been universally reproduced by others [17], leading to some controversy as to the efficacy of such treatments. Howlett *et al.* [18] reported posterior pituitary dysfunction to be common (77%) whilst panhypothyroidism was much less consistent. Conversion of thyroxine to reversed tri-iodothyronine seems to be potentiated by the sympathetic storm which accompanies brain death, and there is also evidence that tri-iodothyronine receptor density declines in association with reduced intracellular tri-iodothyronine [19]. Thyroid function in the donor is thought to be more akin to the sick euthyroid syndrome which, although not usually treated in normal patients, may require intervention in unstable donors for the reasons mentioned above [20]. The cardiovascular actions of tr-iodothyronine have recently been reviewed [21•], and the importance of the extranuclear effects of the hormone on myocardial function highlighted.

Although ADH is not crucial to the maintenance of vascular tone in non-brain dead patients, the absence of vasomotor impulses in brain death would seem to result in a dependency on ADH which has a synergistic action with adrenaline [7].

Following a number of pilot studies, we have developed the regime outlined in Table 1, and have shown that this has a marked effect on donors exhibiting poor or borderline function, whereas donors with good function show little or no benefit [22]. The administration by continuous infusion is critical to the efficacy of the technique.

In a small proportion of donors, the downward spiral of decreasing cardiac output, hypoxia, hypothermia and acidosis can only be broken by mechanical intervention. We have used a modified type of cardiopulmonary support which allows the heart to be unloaded, the circulation to be fully supported, and full oxygenation and the restoration of normothermia to be achieved [23•]. The equipment is easily transportable and uncomplicated to set up and operate.

Hypothermia

Following brain death, there is a loss of hypothalamic temperature control, effectively rendering the donor poikilothermic [24]. Core temperature can only be

Table 1. Hormone replacement therapy

	Dose
Tri-iodothyronine	Bolus; 4μg Infusion; 3μg/h
ADH (Argipressin)	Bolus; 1 U Infusion; 1.5 U/h
Insulin	Infusion to maintain normal blood sugar (minimum 1 U/h)
Adrenaline	Infusion:0–0.5 μg/h, depending on afterload
Hydrocortisone	Bolus; 5μg/kg

maintained above 35°C by the use of active rewarming systems, warmed infusion fluids, warmed and humidified inspired gases and warm ambient temperatures.

Haematological dysfunction

Clotting abnormalities are known to occur in donor patients and their severity may relate to the degree of initial brain destruction [25]. Management involves transfusion of red blood cells, clotting factors and platelets, and the maintenance of normal body temperature.

Management of the donor operation

During the donor operation, multiple organs are usually removed for transplantation. Several medical teams are involved and close co-operation is required. More detailed descriptions of the surgical technique can be read elsewhere [26,27] and the following discussion will concentrate on the points most relevant to the anaesthetist.

Management of the donor during the operative procedure is a continuation of the care commenced at the time of diagnosis of brain death. The principal aim remains that of good perfusion and oxygenation of transplantable organs. To achieve this, close monitoring of the donor is still required. Direct arterial pressure monitoring and the use of a PAFC must continue. The arterial cannula should be sited in the left radial artery and the PAFC in the right internal jugular vein, as the right sub-clavian artery and the innominate vein are ligated early in the operative procedure. Further essential monitoring consists of electrocardiography, pulse oximetry, end-tidal carbon dioxide and urine output. Measurement of core temperature is important, as are regular checks on arterial blood gases, serum electrolytes and glucose, and haemoglobin levels.

The donor operation is a major procedure which may take as long as 6 h. Considerable fluid losses usually occur. Large bore cannulae should be *in-situ*, and pressure infuser bags should be readily available for the rapid administration of replacement fluid. Ideally, 4 units of cross-matched blood should be available for the start of the procedure. The haemoglobin level is maintained at about 10 g/dl to preserve tissue oxygen delivery.

Anaesthesia, by definition, is not required but pressor responses to stimuli and muscular contractions may still be expected. These probably involve intact spinal reflex arcs [28]. The donor is therefore paralysed, and sodium nitroprusside may be required to manage transient hypertension. Blood pressure is usually labile, partly as a result of the loss of vasomotor control but also because of surgical accidental occlusion of the inferior vena cava and intermittent direct stimulation of the adrenal glands. Ventilation is maintained with oxygen and air, or with oxygen and nitrous oxide if medical air is unavailable. Normally the fraction of inspired oxygen should be kept at a level which is required to maintain an arterial PO_2 of about 15 kPa. The arterial PCO_2 should be kept between 4.8 and 5.9 kPa. The surgical procedure involves initial mobilization of the intra-abdominal organs. Once completed, the donor is heparinized with a dose of 3 mg/kg of heparin, prior to cannulation of major vessels. The PAFC should be withdrawn before the superior vena cava is ligated. It is important that the time of aortic cross-clamping is noted as this marks the beginning of the ischaemic period for the transplantable organs [29]. Two major methods of heart–lung preservation are in common use. The first involves placing the donor on full cardiopulmonary bypass with core cooling to 12–15°C, followed by exsanguination and removal of all organs. The second involves the use of a pulmoplegia solution administered directly into the pulmonary artery, with or without a prostaglandin infusion [30]. There is some controversy as to the ideal gas composition for lung inflation, and whether or not to inflate at all, but most groups prefer inflating with room air to about 80% total lung capacity, having initially checked that all lobes have fully expanded.

In multi-organ retrieval the thoracic organs are removed first (en bloc) following simultaneous infusion of preservation fluids to the thoracic and splanchnic organs. If separate heart and lung transplantation is to take place, division is accomplished on the bench. In combined heart/lung/liver transplantation the entire block is removed without division of the inferior vena cava. The liver, pancreas and kidneys (increasingly en bloc) are removed next and further dissection takes place on the bench.

In the UK, there is now a move to organise organ retrieval by coordinated regional multi-organ retrieval teams (Zones), which will do much to minimize the inconvenience to the donor hospital and make for a more efficient system.

Conclusion

Transplantation has become the treatment of choice for selected patients with end organ failure. The shortage of suitable donors has led to waiting list mortal-

ities of up to 40%. Multiple organ donors are clearly a very precious resource and their management requires considerable effort from all those involved. To this end, comprehensive invasive haemodynamic monitoring, including a PAFC, is essential in making objective decisions and optimizing management. For the sake of the recipients, the best standard of care possible should be provided for donors.

References and recommended reading

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- • of special interest
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Transforming the "unacceptable" donor: outcomes from the adoption of a standardized donor management technique. 1995

Transforming the "Unacceptable" Donor: Outcomes from the Adoption of a Standardized Donor Management Technique

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Background: Donor management remains one of the most neglected areas of transplantation. A comprehensive donor management regimen has been developed. The results of the application of this strategy form the basis of this report.

Methods: Full hemodynamic data were collected from 150 multiorgan donors between October 1990 and August 1993. The data were collected at the time of donor team arrival, after insertion of a pulmonary artery floatation catheter and immediately before cardiac excision.

Results: Fifty-two donors (35%) fell well outside our minimum acceptance criteria on arrival. Twenty-one of fifty-two had a mean arterial pressure less than 55 mm Hg (mean 47 mm Hg) despite inotropic support in most cases; 10 of 52 had a central venous pressure greater than 15 mm Hg (mean 18.0 mm Hg); 2 of 52 had a high inotrope requirement greater than 20 μg/kg/min (mean 25 μg/kg/min). After the insertion of a pulmonary artery floatation catheter, an additional 13 of 52 donors were found to have a pulmonary capillary wedge pressure greater than 15 mm Hg (mean 19.8 mm Hg), and the final 6 of 52 had a low left ventricular stroke work index, less than 15 gm (mean 12.8 gm). After optimal management, including hormone replacement 44 of 52 donors yielded transplantable organs (29 hearts, 15 heart and lung blocks). Thirty-seven of forty-four patients (84%) were alive and well from 13 to 48 months after transplantation. There were five early deaths (11%) caused by infection (heart), adult respiratory distress syndrome (heart), arrhythmia (heart), cerebrovascular event (heart and lung), and infection (heart, lung, and liver). Two late deaths (5%) occurred as a result of tamponade (3 months, heart) and infection (14 months, heart and lung). Eight of fifty-two organs were still unsuitable for transplantation after optimum management during the splanchnic dissection as a result of inotrope dependency ($n = 4$), left ventricular hypertrophy ($n = 2$), and coronary artery disease ($n = 2$).

Conclusions: The data indicate that, of the organs which initially fall outside our transplant acceptance criteria, 92% are capable of functional resuscitation. Conversely, superficial assessment may not show compromised function. Optimizing cardiovascular performance also has important implications for the viability of all transplantable organs. This aggressive approach to donor management has resulted in the transplantation of 44 donor hearts that may otherwise have been turned down or inappropriately managed. J HEART LUNG TRANSPLANT 1995;14:734-42.

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bmitted January 3, 1995; accepted February 21, 1995.
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1053-2498/95/$3.00 + 0 14/1/64401

TABLE I Donor physiologic resuscitation regimen

Invasive monitoring
Bolus steroids
Methylprednisolone 15 mg/kg
Insulin (+ dextrose)
Aim for normoglycemia
Minimum 1 U/hr
Arginine vasopressin
1 U bolus
1.5 U/hr
Tri-iodothyronine
4 μg bolus
3 μg/hr

TABLE II Donor heart acceptance guidelines

Mean arterial pressure	> 60 mm Hg
Central venous pressure	< 12 mm Hg
Pulmonary capillary wedge pressure	< 12 mm Hg
Left ventricular stroke work index	> 15 gm
Inotropes	< 5 μg/kg/min

These guidelines are loosely based on the entry criteria for mechanically assisted circulation and are used as an approximate guide for donor heart acceptance. The "marginal" donors referred to in this publication all fell *well outside* these guidelines.

The last two decades have seen tremendous advances in the field of heart transplantation. Before 1980 less than 350 operations were performed in 17 centers with an operative mortality of more than 20% and an overall 1-year survival of 62%. Last year, more than 2500 operations were performed in more than 240 centers with an operative mortality of 9.8% and an overall 1-year survival of 78%, despite the inclusion of higher risk recipients every year.[1]

Although this impressive record has been achieved by significant advances in patient management, and, in particular, improved immunotherapy, further development is now universally limited by the supply of donor organs, which has plateaued over the last few years. The prospect for increasing the referral rate is limited to approximately 15%, and the overall numbers of potential donors is falling, particularly in relation to road traffic accidents.[2]

The major opportunity for improving both the numbers and the quality of donor hearts lies with the most neglected area of transplant medicine, that of donor management. Early graft failure accounts for approximately 26% of acute deaths in heart transplant recipients.[1] In addition there is undoubtedly a significant number of patients who are compromised by poor graft function related to inadequate donor management or preservation.

Following literature reports of the metabolic consequences of brain death in experimental animals[3,4] and later reports of clinical studies,[5] we carried out a number of clinical pilot studies of the efficacy of hormone replacement therapy (HRT) in brain dead donors and showed that donors with compromised cardiac function showed improved function in response to HRT, compared with those donors receiving optimal management without HRT. We were able to confirm that brain death is accompanied by significant reductions in serum free thyroxine, cortisol, arginine vasopressin, and insulin and eventually established a regimen which involves bolus doses of these hormones followed by continuous infusions[6] (Table I). We were further able to demonstrate that this approach can result in the restoration of normal cardiovascular function in the majority of organ donors with previously inadequate function.[7]

Prompted by the increasing shortfall of donor organs, several centers have reported extensions to the classic donor criteria[8] with respect to age,[9] ischemic time,[10] inotropic support,[11] adverse hemodynamics,[12] size mismatching,[13] cause of death,[14-16] and infection.[17] Although some of these experiments have been successful in individual cases, some have not, especially when more than one contraindication is present.[18]

Having taken a considerable interest in this problem, it has become clear that comprehensive hemodynamic monitoring is essential to making an objective assessment of function and for guiding optimum management.[19] Failure to provide adequate physiologic support to potential donors accounts for at least 25% of lost donor organs.[20,21] Adopting an aggressive approach to donor management has allowed us to increase our donor retrieval rate by approximately 30% without prejudicing outcome. We suggest that this provides one of the few remaining methods for safely increasing the donor pool.

PATIENTS AND METHODS

Between October 1990 and October 1993, 150 multiorgan donors were fully instrumented during

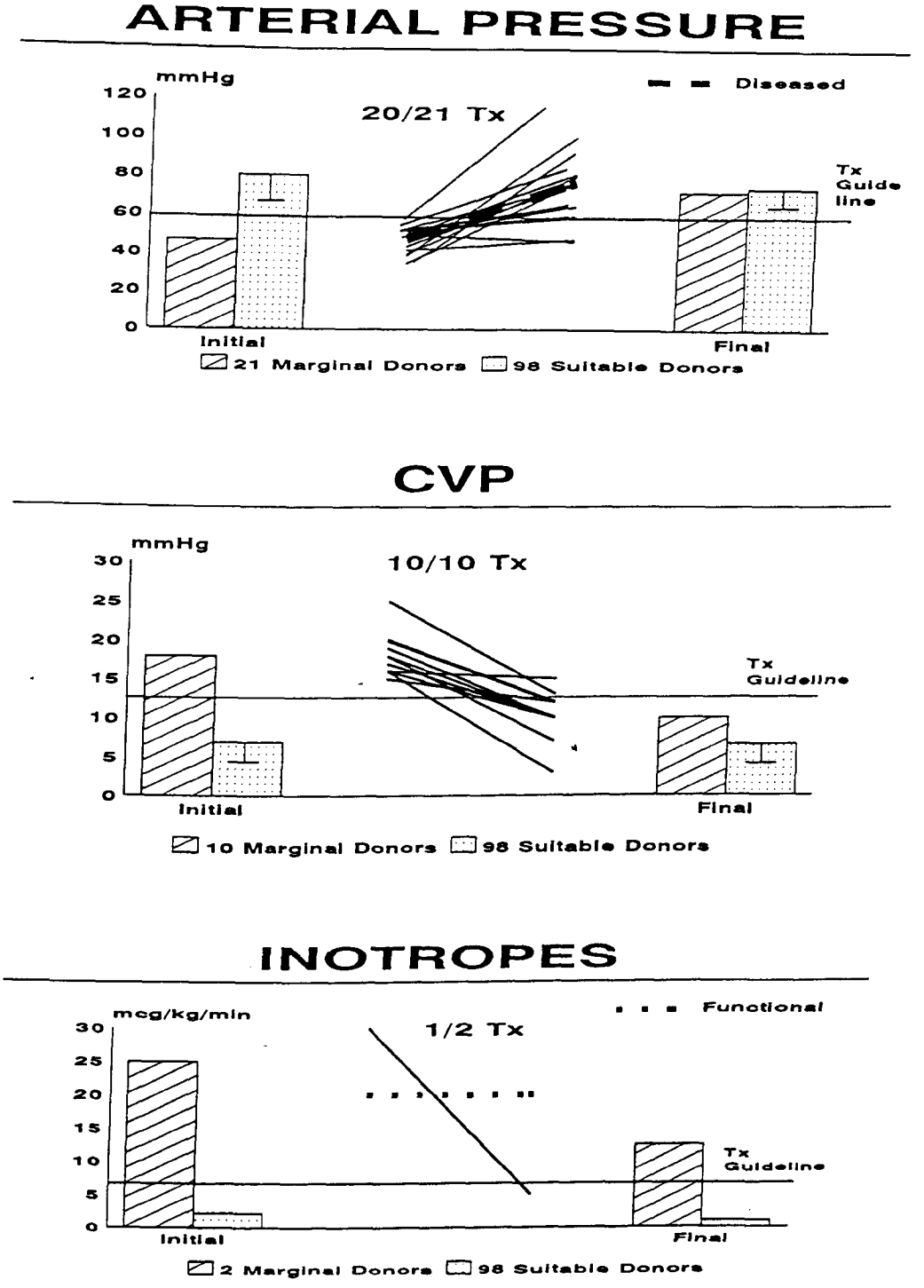

FIGURE 1 Mean and individual measurements of arterial pressure, central venous pressure *(CVP)*, and inotropic requirements of the 33 donors unacceptable on initial inspection compared with the 98 initially suitable donors. *Broken lines* indicate organs not transplanted. *Tx*, Transplantation.

the retrieval operation. This involved transport to the donor hospital of our own compact monitoring equipment and the institution of invasive arterial, internal jugular venous, and Swan-Ganz pulmonary artery catheterization (Swan-Ganz catheter; Baxter Healthcare Corporation, Edwards Div., Santa Ana, Calif.) as soon as the donor arrived in the operating room. Ventilation, fluid replacement, and electrolytes were optimized, and the first set of measurements were undertaken while the cardiac surgeon performed a median sternotomy and visually examined the thoracic organs. During this inspection a

TABLE III Hemodynamic profiles of the 52 initially *unacceptable* donors

Patient No.	AoP	RAP	Inotropes	PCWP	LVSWI	Not used	Deaths
1	**52**	8	0	10	12.8		CVA day 16
2	**51**	6	0	9	15.5		
3	**53**	5	0	9	18.7		
4	**52**	1	0	4	15.1		
5	**53**	1	5	4	36.7		
6	**35**	6	5	7	13.8		
7	**39**	6	0	7	13.9		
8	**48**	6	2.5	10	19.6	LVH	
9	**50**	15	5	17	6.1		Infection day 16
10	**50**	2	0	5	6.5		
11	**42**	5	14	3	14.9		
12	**40**	0	4	2	20.1		
13	**44**	0	12	7	16.2		
14	**49**	3	5	1	21.2		
15	**54**	12	2.5	8	18.9		
16	**51**	4	3.0	8	35.6		
17	**53**	9	5	11	18.9		
18	**46**	1	5.5	2	18.9		
19	**48**	0	0	3	33.6		
20	**50**	4	2.5	6	22.5		
21	**26**	1	15	7	7.9		
22	85	**15**	0	15	50.0		
23	60	**16**	8	19	22.1		Tamponade 3 mo
24	81	**17**	6.5	20	39.4		
25	62	**18**	5	23	15.6		Infection 14 mo
26	114	**16**	0	20	53.4		
27	60	**25**	5	11	19.0		
28	79	**19**	8	19	27.5		
29	62	**20**	6	20	28.9		
30	65	**16**	16	9	18.9		
31	112	**18**	4	24	49.2		
32	56	9	**20**	7	27.2	Inotrope dep.	
33	60	1	**30**	11	67.1		
34	55	7	0	**22**	20.2		
35	140	11	0	**17**	50.3		
36	71	13	6	**20**	16.3	LVH	
37	80	13	0	**19**	34.8		
38	117	9	0	**17**	96.6		
39	86	10	0	**18**	24.3		
40	92	10	5	**23**	42.3		ARDS day 1
41	65	9	10	**24**	26.1		
42	76	11	12	**17**	26.3	Inotrope dep.	
43	75	10	6	**16**	19.5	CAD	
44	66	14	7	**22**	38.4	CAD	
45	81	8	2.5	**25**	15.7	Inotrope dep.	
46	84	6	0	**18**	21.5		
47	63	7	20	20	**10.1**		Arryhthmia 12 days
48	62	6	3	13	**14.4**		
49	65	10	2.5	11	**11.0**		
50	72	10	0	11	**13.5**	Inotrope dep.	
51	78	3	2.5	6	**13.3**		
52	62	12	0	14	**14.9**		

Donors were in *unacceptable* group if they fell *well outside* acceptance guidelines (Table II) in the order that measurement data became available—initially without Swan-Ganz catheter data (Baxter Health Care) and later including comprehensive data. Cut-off points were: mean arterial pressure < 55 mm Hg, central venous pressure (CVP) and pulmonary capillary wedge pressure (PCWP) > 15 mm Hg, inotropes > 20 μg/kg/min, left ventricular stroke work index (LVSWI) < 15 gm. Primary categorization points are highlighted. *AoP*, Aortic pressure; *RAP*, right atrial pressure; *CVA*, cerebrovascular accident; *LVH*, left ventricular hypertrophy; *dep.*, dependent; *ARDS*, acute respiratory distress syndrome; *CAD*, coronary artery disease.

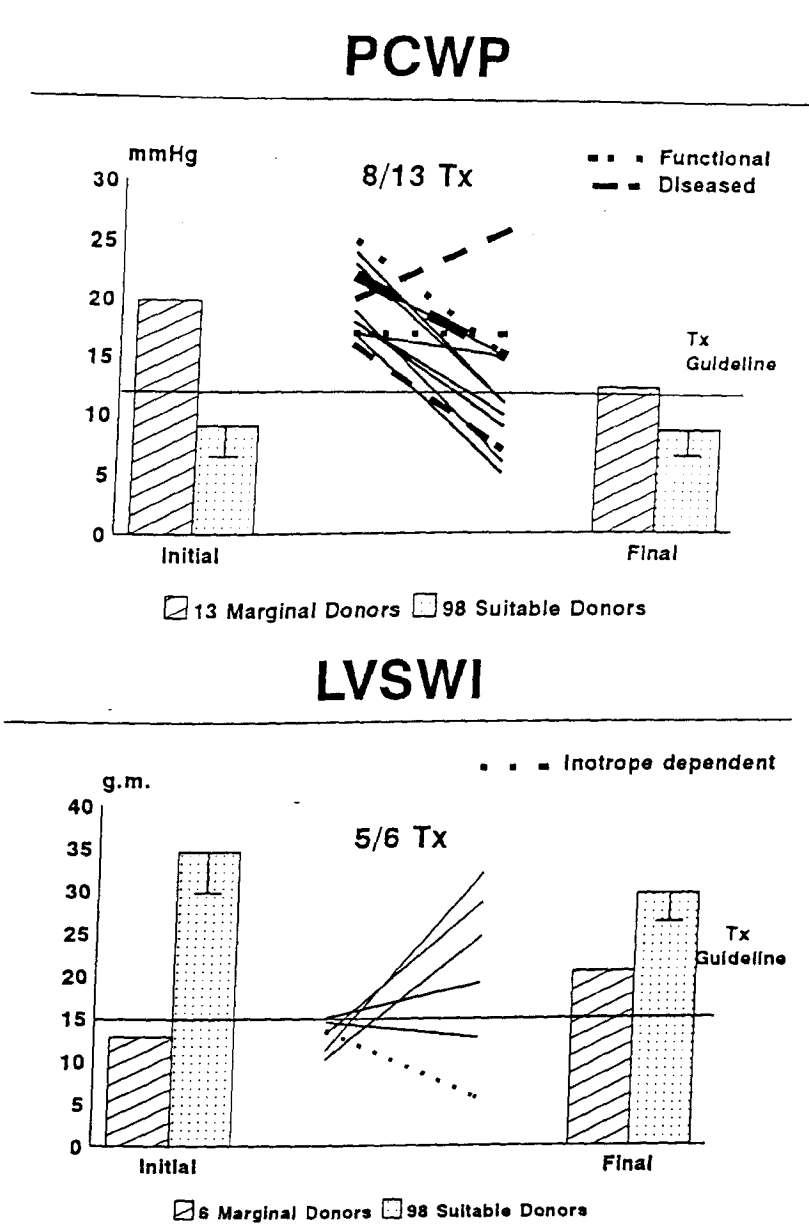

FIGURE 2 Mean and individual measurements of pulmonary capillary wedge pressure *(PCWP)* and left ventricular stroke work index *(LVSWI)* of the further 19 unacceptable donors after full monitoring compared with the 98 initially suitable donors. *Broken lines* indicate organs not transplanted. *Tx,* Transplantation.

TABLE IV Outcomes for the 98 initially suitable donors

	Donors accepted
After inspection	98/150
Transplanted	89/98
Not transplanted	9/98
Coronary artery disease	4/9
Inotrope dependent	3/9
Right heart failure	1/9
Lignocaine poisoning	1/9

comprehensive set of measurements were performed and HRT was commenced. During the splanchnic dissection further sets of measurements were taken, as a guide to management. Once the splanchnic dissection was completed, and, with the cardiac surgeon back at the table, the final set of measurements were taken as a basis for donor organ acceptance. A detailed description of our donor management regimen has been published elsewhere.[19]

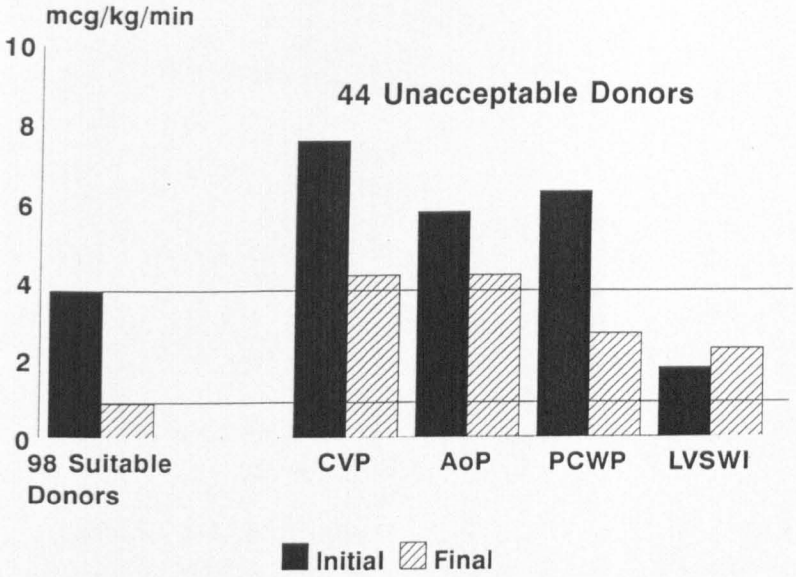

FIGURE 3 Changes in inotropic requirements by group according to functional impairment compared with the 98 initially suitable donors, 63% of the entire group required a mean inotropic dose of 6.8 μg/kg/min which reduced to 45% requiring a mean dose of 4.8 μg/kg/min by the end of the procedure. *CVP,* Central venous pressure; *AoP,* aortic pressure; *PCWP,* pulmonary capillary wedge pressure; *LVSWI,* left ventricular stroke work index.

TABLE V Survival and causes of death in the 89 recipients of hemodynamically suitable donors

Suitable donors	
Alive and well 68/89 (76%)	13-48 mo after transplantation
30-day mortality 11/89 (12%)	
3 Elevated PVR	Heart
3 graft failure	1 Heart, 2 heart/lung
1 Pancreatitis	Heart/lung
1 Infection	Heart/lung
1 CVA	Heart
1 Arrhythmia	Heart
1 Pulmonary embolus	Heart
Late deaths 10/89 (1%)	
2 CVA 7 and 25 mo	Heart and heart/lung
1 Pancreatitis 3 mo	Heart
4 Coronary disease 5, 7, 9, 16 mo	Heart
1 Malignancy 4 mo	Heart
1 Multiorgan failure 3 mo	Heart/lung
1 Infection 4 mo	Heart/lung

PVR, Pulmonary vascular resistance; *CVA,* cerebrovascular accident.

TABLE VI Survival and causes of death in the 44 recipients of initially *unacceptable* donors

Alive and well 37/44 (84%)	13-48 mo after transplantation
30-day mortality 5/44 (11%)	
Arrythmia	Heart
Infection	Heart
Acute respiratory distress	Heart
Cerebrovascular event	Heart and lung
Infection	Heart/lung and liver
Late deaths 2/44 (5%)	
Tamponade 3 mo	Heart
Infection 14 mo	Heart and lung

Of the 150 multiorgan donors, 133 yielded transplantable thoracic organs; 87 heart and 46 heart and lung blocks, together with splanchnic organs.

There were 52 of 150 donors falling well outside our acceptance guidelines (Table II). On initial inspection, 33 of 52 donors were judged to be "unacceptable": 21 on the grounds of low mean arterial pressure (mean 47 mm Hg), 10 on the grounds of a high central venous pressure (mean 18 mm Hg), and 2 requiring high inotropic support (25 μg/kg/min). Figure 1 shows the individual and mean values at the start and end of the procedure for these groups, compared with the mean values for the remaining donors.

With full monitoring results, an additional 19 of 52 donors fell well outside our acceptance guidelines (Table III): 13 on the grounds of high pulmonary

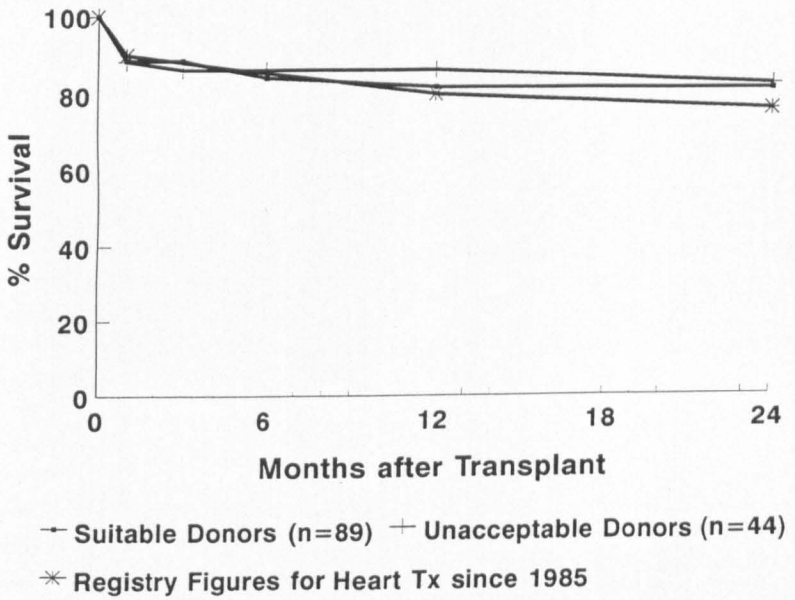

FIGURE 4 Comparative actuarial survival curves for recipients of the marginal and suitable organs, compared with the International Society for Heart and Lung Transplantation Registry figures since 1985. *Tx,* Transplantation.

capillary wedge pressure (mean 19.8 mm Hg) and six on the grounds of a low left ventricular stroke work index (12.8 gm). Figure 2 shows the individual and mean values at the start and end of the procedure for these groups, compared with the mean values for the remaining donors. In the 13 patients with high pulmonary capillary wedge pressures, a significant left/right imbalance was observed: mean central venous pressure of 10.2 mm Hg with a mean pulmonary capillary wedge pressure of 19.8 mm Hg, which would not have been detected without full monitoring. Table III gives full hemodynamic profiles of all of the donors in the unacceptable group.

An improvement in function was seen in 48 of 52 of these donors, which brought them back within our guidelines. Figure 3 shows the changes in inotrope support for the entire group, together with each of the unacceptable groups. Overall, 63% of donors initially required inotropic support with a mean dose of 6.8 μg/kg/min; this percentage was reduced to 45% of donors, requiring a mean dose of 4.8 μg/kg/min, at the end of the retrieval procedure. However, four donors remained inotrope dependent. An additional four hearts were turned down on the grounds of cardiac disease, despite acceptable function: two with palpable coronary disease and two with significant ventricular hypertrophy. Our management regimen resulted in 44 initially marginal hearts being successfully transplanted.

Of the 150 donors, 98 were within or close to our hemodynamic criteria on inspection. Of these, nine were not transplanted: four were rejected on the grounds of significant coronary artery disease, three became inotrope dependent, one developed right heart failure during the procedure, and one was poisoned with an overdose of lignocaine (Table IV). Accordingly, only 5% overall were refractory to our management regimen.

RESULTS

The overall survival in the group of 89 recipients who received organs from initially acceptable donors was 76% over a follow-up period of 13 to 48 months. There were 21 deaths; 11 (12%) of these were early: three from an elevated pulmonary vascular resistance, one from graft failure (heart), two from graft failure (lung), one from arrhythmia, one from infection, one from pancreatitis, one from a pulmonary embolus and one from a cerebrovascular accident. There were 10 (11%) late deaths (Table V).

Of the 78 of 89 patients surviving to discharge, there were 52 heart and 26 heart and lung recipients. The overall survival in the group of 44 recipients who received hearts from unacceptable donors was 84% over the same follow-up period of 13 to 48 months. There were five (11%) early deaths: one from an arrhythmia (heart), two from infection (heart and heart-lung and liver), one as a consequence of acute respiratory distress syndrome (heart), and one from a cerebrovascular accident (heart and lung). There were an additional two (5%) late deaths: one from

tamponade (heart) at 3 months and one from infection (heart and lung) at 14 months (Table VI). As is evident from the previously noted list, only one of these deaths could be directly attributed to a cardiac cause. These results compare well with those of the International Society for Heart and Lung Transplantation Registry 30-day mortality figures of 9.8% (hearts) and 21% (heart and lung) and 1-year Registry mortality figures of 78% (hearts) and 60% (heart and lung).[1] Actuarial survival curves show no statistical differences between our two groups and the Registry heart transplant survival since 1985 (Figure 4).

DISCUSSION

The donor management regimen described in this article has been developed over the past 4 years. This regimen was originally inspired by the Cape Town group,[5] with subsequent influence by publications from Japan with respect to the key role of vasopressin.[22] The importance of changing the focus of management from that of minimizing the effects of cerebral trauma to that of optimal cardiovascular support has been recognized elsewhere,[23,24] but a crucial change in our own practice has been the inclusion of a cardiac trained anesthetist as a member of the donor team. The use of continuous infusions of HRT has also been shown to be important. Despite our best efforts, however, a small number of donors (5% in this study) were still refractory to this management regimen. We have recently investigated the potential for physiologically resuscitating these donors by means of normothermic support bypass—with some success.[26]

Given the interactive nature of preload, afterload, and inotrope therapy on cardiac function, it is difficult to find a single comprehensive descriptor. However, in this study, marginal donors have been differentiated on the basis of wide deviation from our general acceptance criteria despite being classified according to the first major condition by which they fail (Table III). We do not seek to delineate the effects of HRT from optimal cardiovascular management with this report because our previous studies have already provided us with sufficient evidence of the efficacy of HRT in donors with compromised cardiac function.

The most important factor to emerge from our experience has been the necessity to use comprehensive monitoring which provides objective data on which to base optimal donor management and objective assessment of cardiac function. This monitoring reduces the risks of retrieving unsatisfactory organs or the exclusion of potentially satisfactory organs.

The specific interventions, outlined previously, provide a mechanism for improving perioperative cardiovascular function, which has a particular relevance to the quality of retrieved donor hearts but has important implications for the viability of all transplantable organs. This study suggests that the numbers of satisfactory donor hearts may be increased by up to 30% without compromising recipient outcome. Overall perioperative cardiovascular function is improved providing the opportunity for increased viability and function of all transplantable organs.

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D

Oxygenated crystalloid cardioplegia: a new technique. 1989

Perfusion 1989; 4: 297–301

Oxygenated crystalloid cardioplegia: a new technique

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Introduction

Numerous experimental and clinical studies have demonstrated that the oxygenation of cardioplegic solutions is beneficial since although hypothermic cardioplegic arrest provides a marked reduction in myocardial oxygen demand, there is still a small but finite consumption of oxygen and substrate.[1–8]

In an effort to meet these metabolic demands the use of blood-based cardioplegic solutions has gained some popularity over recent years and more recently still, systems for oxygenating crystalloid cardioplegic solutions have been developed by several manufacturers. Whilst most of the delivery systems are effective, they are both complex and expensive. Analysis of replies to a recent perfusion safety survey revealed a disturbing increase in the number of serious perfusion accidents related to cardioplegic delivery systems.[9]

We describe a novel technique for the oxygenation of crystalloid cardioplegic solutions which allows control of oxygen content whilst remaining both simple and inexpensive.

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Method

One litre Viaflex bags of Ringer's solution are placed in a warming oven (45°C) overnight. This causes the nitrogen content to fall from approximately 2.3 vols% to 1.1 vols%. The air which has been driven out of the solution is removed by needle and syringe and the cardioplegia concentrate added to the Ringer's solution. Pure oxygen is then added via a filter (Millipore 0.2 microns) to a pressure according to the required oxygen content and the Viaflex bag is refrigerated at 4°C.

From Figure 1 it is evident that there is a steep increase in pO_2 related to the internal pressure, up to about 55mmHg, after which there is little further increase in oxygen content with increasing oxygen pressure. Solutions prepared in this way have been shown to be stable for more than 20 days. In addition, the pH remains unaltered in bicarbonate buffered solutions.

Using the same technique without prewarming results in a lower oxygen content (3.5 vols% compared with 5.4 vols%) and the application of external pressure (400mmHg) can be used to speed up the equilibrium (Figure 2).

Cardioplegia delivery is then achieved by simple external pressurization of the Viaflex bag. The only precaution required is to keep the bag cold between infusions when using higher oxygen content solutions, since oxygen tends to come out of solution with rewarming. The use of a hydrophilic-hydrophobic cardioplegia filter provides additional security.

Discussion

The oxygen demands of the nonworking heart (vented and beating or fibrillating) have been reported to be approximately 4ml/min/100g at 37°C, falling to 0.5ml/min/100g at 4°C. The use of hyperkalaemic cardioplegia reduces this demand to 1.5ml/min/100g at 37°C and 0.15ml/min/100g at 4°C (Figure 3).[8]

Although blood-based cardioplegic solutions are capable of transporting more oxygen than crystalloid solutions, oxygen delivery is compromised by the conditions under which it is used. Hypothermia, a high pH and reduced red cell 2-3 diphosphoglycerate all increase the affinity between haemoglobin and oxygen to the extent that below 20°C, most oxygenated crystalloid solutions will deliver more oxygen than blood-based solutions.[10] There may, however, be other benefits from using blood as a cardioplegic base, not related to its oxygen carrying capacity, but these are as yet unclear.

From Figures 3 and 4 it is evident that the oxygen demand of a 250g chemically arrested heart at 15°C (approximately 50ml of oxygen/hour) could be met by 21ml of crystalloid cardio-

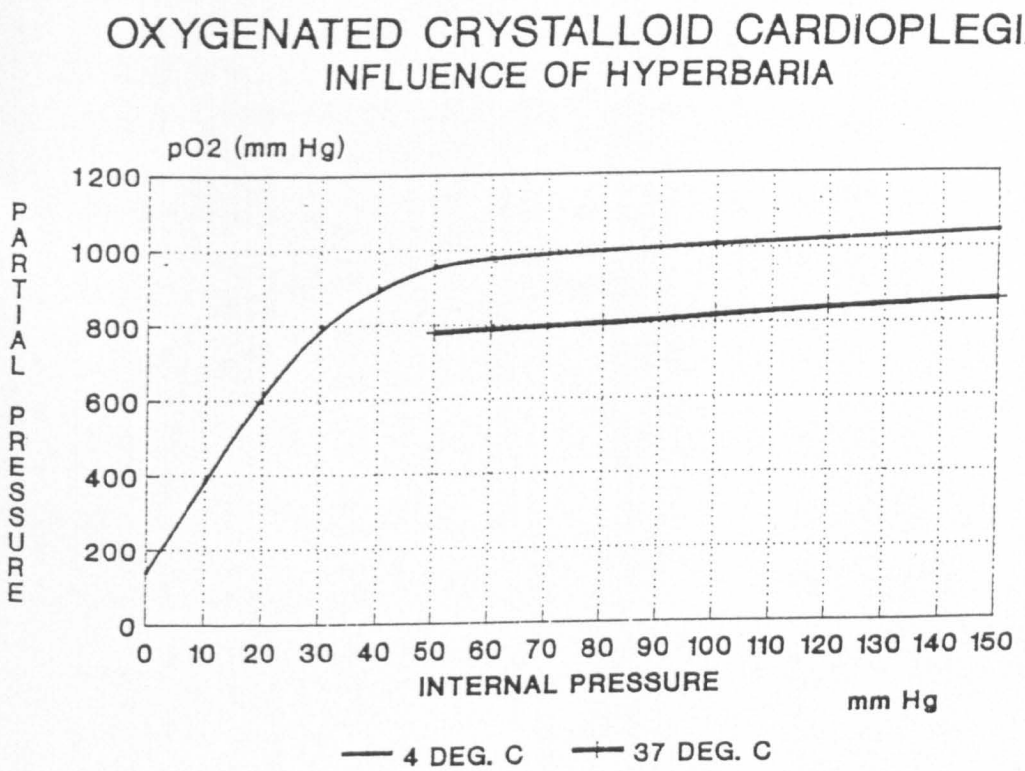

Figure 1 Oxygen tensions produced as a result of prewarming Ringer's solution overnight followed by the addition of oxygen at pressures between 0 and 150mmHg. Following the addition of oxygen, the Viaflex bag is refrigerated at 4°C for 24 hours. Oxygen tensions were measured at 37°C on an IL blood gas analyser (613). This means that PO_2 measurements tended to be lower than the actual tension, particularly at high levels of oxygenation. Tensions at 4°C are extrapolated between loading pressures of 50mmHg and 150mmHg. The actual tension to which the myocardium is exposed will fall somewhere between these two curves, depending on the temperature.

plegia at a PO_2 of 260mmHg, 15ml at a PO_2 of 560mmHg and 9.8ml at a PO_2 of 960mmHg. The volume and frequency of cardioplegia delivery can therefore easily be calculated.

A number of elegant cardioplegia delivery systems are available, but they are all relatively complex and expensive. A recent comparative study of four such systems suggested that a bubbler oxygenating system has distinct advantages over a membrane system based largely on differences in the ability to deliver solutions at 4°C. Oxygen carrying capacity for crystalloid solutions was 0.56 vols% for nonoxygenated, 3.2 vols% for bubbler oxygenated, 3.7 vols% for membrane oxygenated and for the blood cardioplegia 11.3 vols% with a calculated availability of 4.2 vols% at 12°C.[11]

The use of bubbler oxygenated systems has been associated with calcium paradox due to carbon dioxide washout and the resultant alkalinity. It may therefore be prudent to add calcium to these solutions and to ventilate with 5% CO_2.[12]

The system which we describe avoids the technical and chemical complications described above. Oxygen contents of up to 5.4 vols% are readily obtainable and intermediate contents are also easily achieved. Lastly, the system has the overwhelming advantages of extreme simplicity, low cost and intrinsic safety.

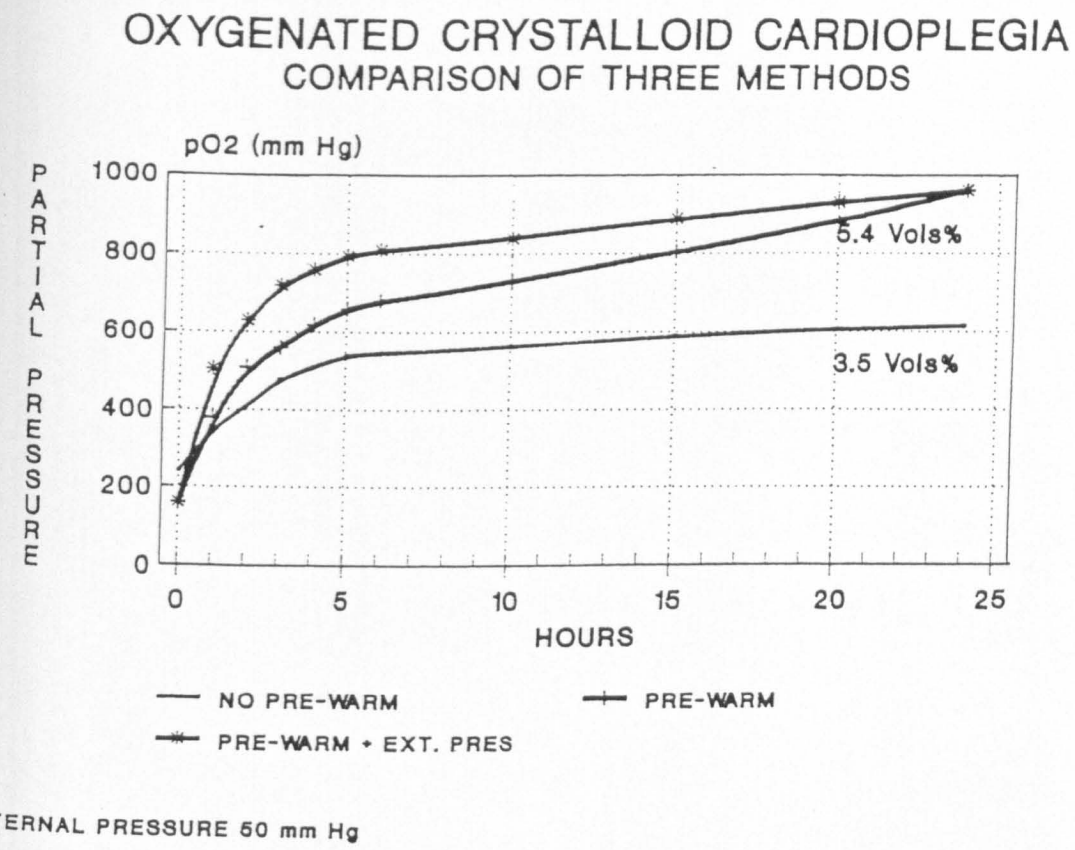

Figure 2 Time taken to reach a steady oxygen tension following loading with oxygen to a pressure of 50mmHg with and without prewarming and removal of air. Equilibrium can be speeded up by the application of external pressure to the Viaflex bag (400mmHg). Removal of approximately half of the dissolved air makes a significant difference to the final oxygen content of the solutions.

Acknowledgements

This work is supported by a grant from The British Heart Foundation.

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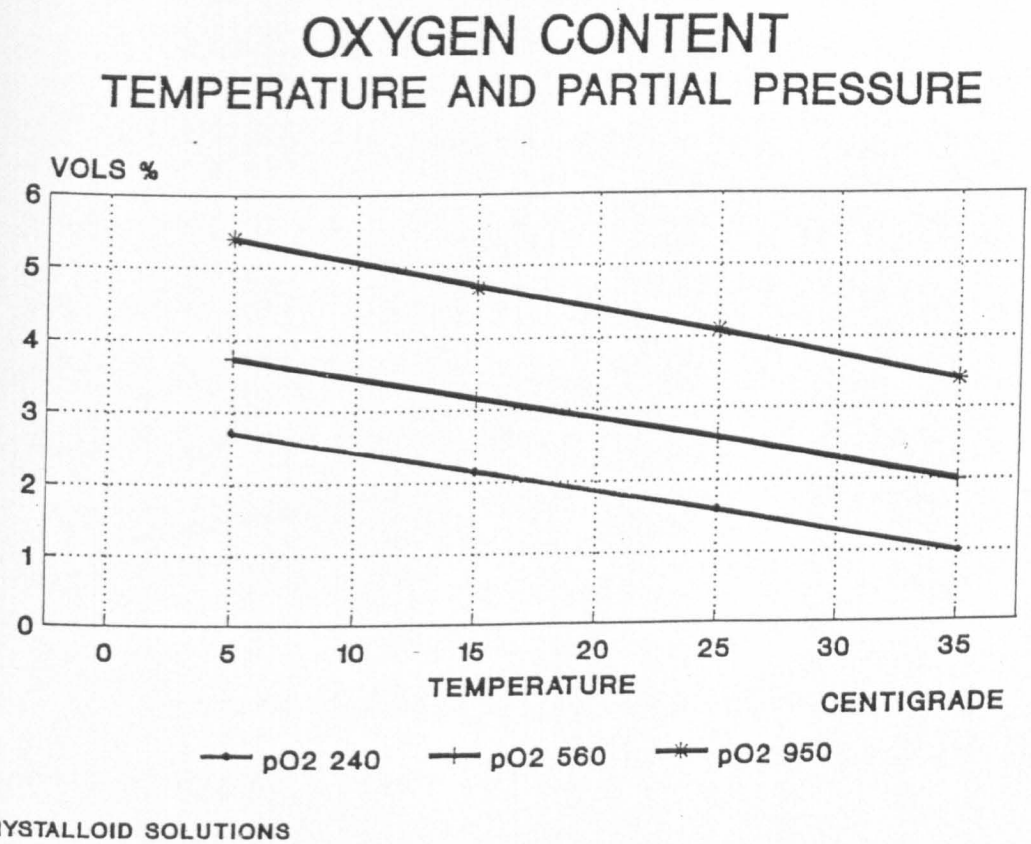

Figure 3 Oxygen content of crystalloid solutions at three different tensions between 0°C and 35°C.

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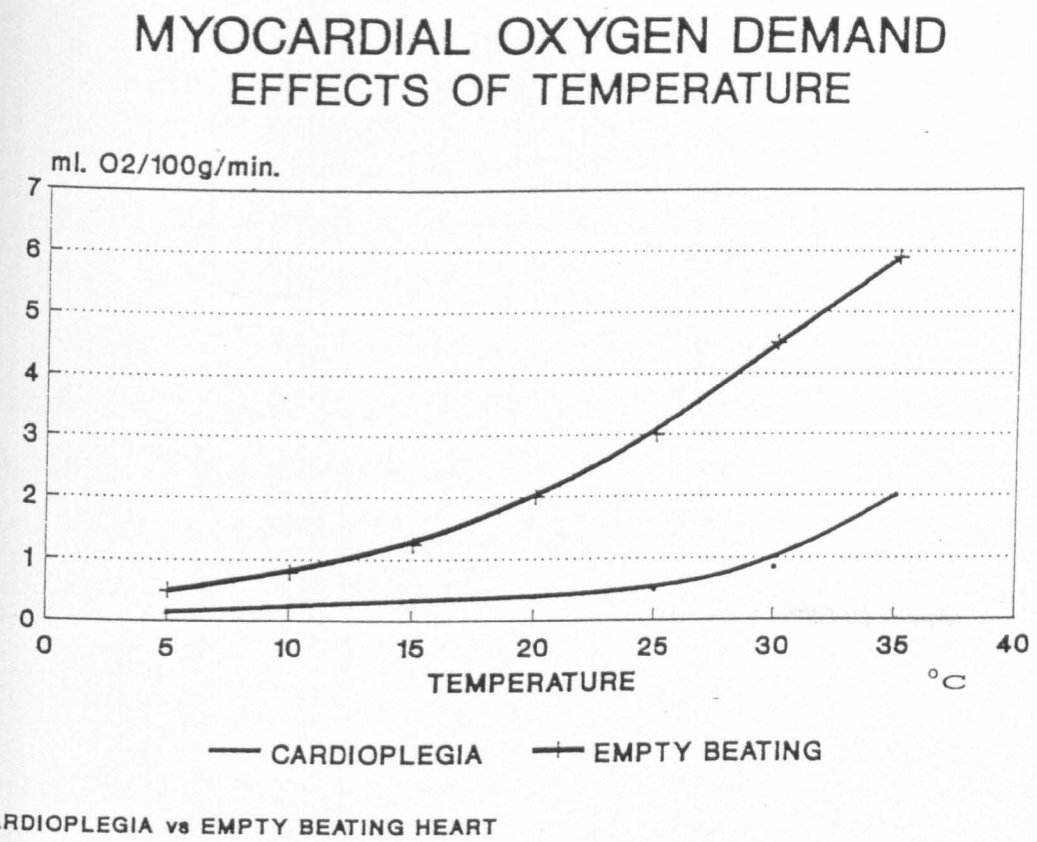

Figure 4 Differences in myocardial oxygen demand at temperatures between 37°C and 5°C. The use of hyperkalaemic cardioplegia produces a significant reduction when compared with an empty beating or fibrillating heart.

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E

Multi-organ donor resuscitation. 1992

Perfusion 1992; **7**: 213–216

Multiorgan donor resuscitation

DR Wheeldon, CD Potter, F Ciulli, J Dunning, S Gray, A Oduro, J Wallwork and **S Large** Papworth Hospital, Cambridge

Introduction

Approximately 30% of donor referrals for cardiac transplantation are refused on grounds of 'medical unsuitability', predominantly because of apparently poor haemodynamic function, notwithstanding significant inotropic support.

Following pilot studies on methods of reversing the deleterious metabolic consequences of brain death, during the past 14 months we have been able to restore normal haemodynamics in 14 of 61 fully instrumented (Swan Ganz) donors who had unsatisfactory cardiorespiratory function on initial evaluation (CI < 2.0 l/min/m^2, PCWP > 12 mmHg, inotropes > 5 mcg/kg/min against an afterload of 800–1200 dynes·sec·cm^{-5}). A hormone package comprising T_3, ADH and insulin was administered as a continuous infusion during the two to three hour donor organ retrieval procedure. These 14 pharmacologically 'resuscitated' hearts showed a mean post-treatment increase in LVSWI of 73% compared with a decrease of 25% in untreated donors.

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We believed that donors refractory to pharmacological resuscitation might respond to haemodynamic resuscitation on a form of cardiopulmonary support (CPS), since this would allow the downward spiral of hypoxia, hypothermia and cardiac decompensation to be broken whilst providing stability during the splanchnic dissection. Bypass has been used by a number of transplant teams as a method of total body cooling for heart-lung organ retrieval[1] and was used by Barnard to support the first clinical heart donor[2] but has not previously been reported as a method of donor resuscitation. We report on what we believe to be the first such use of CPS.

Case report

Following a road traffic accident a 21-year-old male sustained a dislocated hip and subdural haematoma leading to diffuse cerebral oedema and subsequent brain stem death seven days later. During his treatment he received large volume infusions of crystalloid and colloid, leading to a positive fluid balance of 2.5 litres on day seven,

Table 1 Perioperative haemodynamic changes. The table shows the changes in haemodynamics and inotropic support from admission to the operating theatre. The hormone package brought about some improvement after one hour of therapy, but function was still unacceptable. CPS values were obtained off bypass at the intervals shown.

	Theatre	One hour post hormones	70 min CPS	170 min CPS	One hour post transplant
AoP (mmHg)	31	42	52	67	73
CVP (mmHg)	19	11	7	4	6
PA (mmHg)	21	27	15	17	24
PCWP (mmHg)	21	20	6	7	12
CO (l/min)	6.6	14.2	6.9	6.8	7.6
CI ($l/min/m^2$)	3.4	7.4	3.6	3.5	4.0
SVR ($dynes \cdot sec \cdot cm^{-5}$)	145	174	525	734	706
LVSWI (g·m)	5.3	18.7	19.0	29.2	33.2
ADH (IU/h)	2	6	12	9	–
Dobutamine	40	40	–	–	–
Dopamine	5	5	5	5	5
Noradrenaline	–	–	–	–	0.05
Isoprenaline	–	–	–	–	0.003
Urine output (ml)	0	0	120	460	–

AoP = aortic pressure; CVP = central venous pressure; PA = pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; CO = cardiac output; CI = cardiac index; SVR = systemic vascular resistance; LVSWI = left ventricular stroke work index; ADH = Anti-diuretic hormone. Values for all inotropes are in mcg/kg/min.

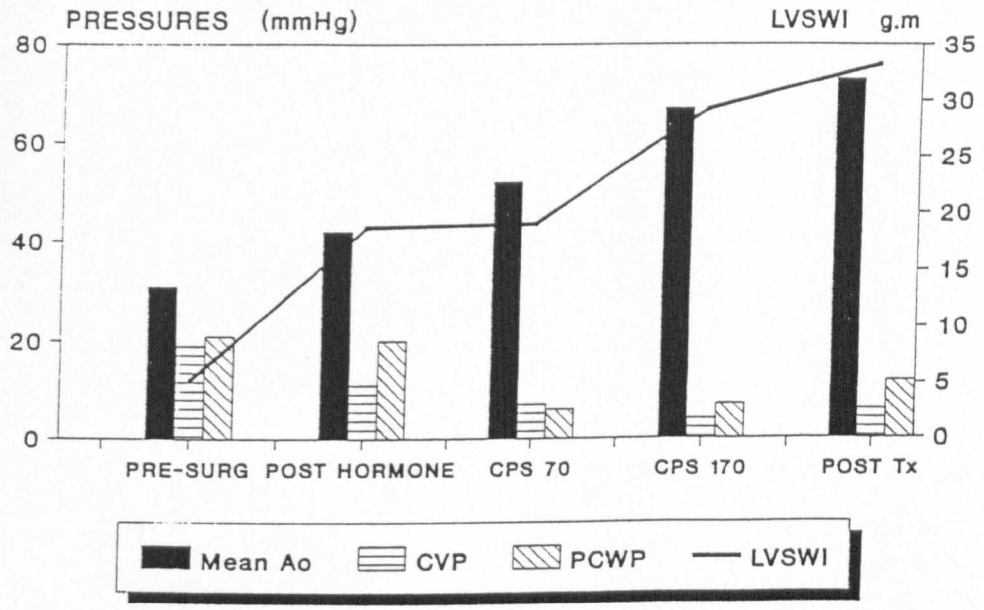

Figure 1 Graphical representation of key parameters improvements following hormone therapy followed by CPS. LVSW has been shown by our group and others to offer the best single indicator of cardiac function.

a decreasing urine output and blood pressure requiring increasing inotropic support. Relatives gave permission for multiorgan donation. The ethical aspects of using advanced methods for improving the quality of donor organs was discussed with our Ethical Committee.

On transfer to theatre his haemodynamics revealed a picture of cardiogenic shock despite significant inotropic support (Table 1), and he had been anuric during the previous six hours.

Splanchnic dissection revealed an ischaemic bowel with a blue, congested liver. Cardiac inspection showed a distended, poorly contracting heart. Treatment with our hormone package was commenced and although a significant improvement in haemodynamics was achieved following one hour of treatment (Figure 1), function was still unacceptable. It was decided to place the donor on CPS. A CPS circuit (Medtronic Inc) (Figure 2), comprising a hollow-fibre membrane oxygenator with integral heat exchanger and centrifugal blood pump was primed with 800 ml of Plasmalyte A and connected to the donor via a single right atrial cannula and high aortic

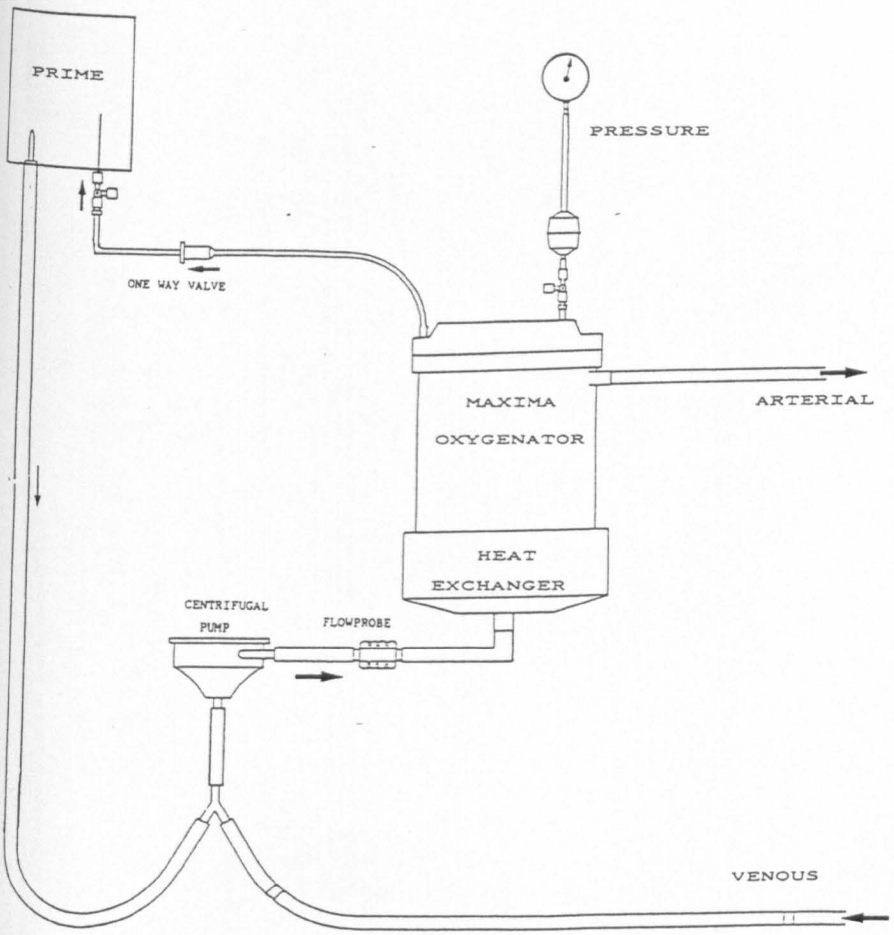

Figure 2 CPS circuit. The circuit used was an adaptation of the Medtronic closed chest system. The prime one litre bag was used as a reservoir during priming and de-airing and thereafter as a buffer reservoir.

cannulation. The blood flow was adjusted so as to unload the heart whilst still providing pulsatile arterial pressure. Blood gases and electrolytes were normalized with normothermic perfusion at an Index of 1.5 to 1.8 l/min/m^2 over the intervening three hours. There was a rapid improvement in cardiac contractility and in the ischaemic appearances of the splanchnic vascular bed, together with a resumption in urine output after 20 minutes of CPS. After 70 minutes CPS was weaned and haemodynamic function again evaluated. Further improvements were noted (Figure 1) but it was decided to continue CPS for a further two hours. During this period it had been possible to withdraw all the dobutamine support, normalize biochemistry and blood gases, restore normal urine output and unload the heart. Final haemodynamic assessment demonstrated very acceptable cardiac function. An early decision was made not to use the liver for transplantation although a biopsy taken post-CPS was reported as normal. Both kidneys functioned exceptionally well following transplantation and post-transplant cardiac function was normal.

Discussion

We believe that this case demonstrates one of the few remaining methods of increasing the donor pool whilst providing a means of improving organ function of all transplantable organs. Data from the Registry of the International Society for Heart and Lung Transplantation reveal that primary graft dysfunction still accounts for 26% of post-transplant mortality.[3]

Modern bypass equipment makes the application of such a technique a logistical reality in terms of transport of equipment to donor hospitals, and we believe that the more extensive application of this technique could yield improved post-transplant organ function and make a significant impact on current transplant mortality and morbidity.

Acknowledgements

This work was supported by The British Heart Foundation.

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F

Functional assessment and management of heart donors: a rationale for characterization and a guide to therapy. 1995

Functional Assessment and Management of Heart Donors: A Rationale for Characterization and a Guide to Therapy

C. D. O. Potter, BA, D. R. Wheeldon, MIBiol, and
J. Wallwork, BSc, MBChB, FRCS(E), MA

Background: Traditional methods for the functional evaluation of a donor heart have relied on superficial hemodynamic data and visual inspection of the action of the heart at sternotomy. The International Registry has continued to report significant mortality for heart transplant recipients from primary graft dysfunction that may be due to donor management, donor organ selection, organ preservation, or recipient factors. The literature reports the loss of at least 25% of potential donors because of the provision of inadequate physiologic support.

Methods and Results: We have now spent several years in establishing and refining a strategy for optimizing donor management, which has resulted in the safe expansion of our donor pool by approximately 30%. Central to this management regimen has been the use of comprehensive perioperative invasive monitoring used by a cardiac anesthetist who takes responsibility for donor management during the retrieval operation.

Conclusion: This article outlines the technique which has evolved for the functional evaluation of a donor heart, which is now used in our institution as a guide to management and as a basis for decision making regarding organ suitability. J HEART LUNG TRANSPLANT 1995;14:59-65.

Two important aspects of cardiovascular function have to be borne in mind with any physiologic analysis. The first is that the circulation consists of two separate pumps and resistances linked in series, the outputs of which must necessarily be the same for equilibrium to be maintained. The second is that because flow, volume, and generated pressures are interactive, analysis in more than one dimension is required for adequate interpretation. In addition, it is necessary to characterize the system by making measurements at a minimum of two different points, by adjusting preload, inotropy, or afterload. This implies measuring both right-sided and left-sided heart pressures and cardiac output (CO), and making the relevant calculations. Adjustment to preload, inotropy, or afterload can then be made in the appropriate direction and the measurements repeated.

The techniques described in this article are based on nomograms, constructed from basic principles of physics. The nomograms detail the interaction between the left and right ventricles and display the trend and current hemodynamic status of the donor and thus highlight the effect of individual interventions. The technique is easily instituted in any patient undergoing pulmonary artery CO catheterization and arterial blood pressure monitoring. Similar function charts for the heart have been described in the past by Van den Horn et al.,[5] but these have been related to animal experimentation. They have concentrated on left ventricular performance only, with an emphasis on maximum efficiency versus the optimum working point for an individual heart.

From the Transplant Unit, Papworth Hospital, Papworth Everard, Cambridge, United Kingdom.
Poster presented at the Fourteenth Annual Meeting and Scientific Sessions of the International Society for Heart and Lung Transplantation, Venice, Italy, March 23-26, 1994.
Submitted March 16, 1994; accepted August 8, 1994.
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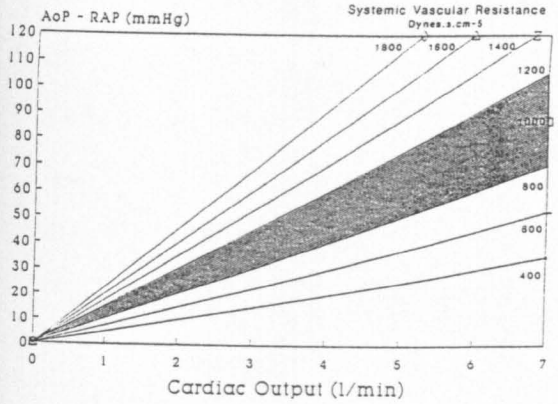


FIGURE 3 Target systemic vascular resistance. *Shaded section* indicates target area. *AoP,* Mean arterial pressure; *RAP,* right atrial pressure.

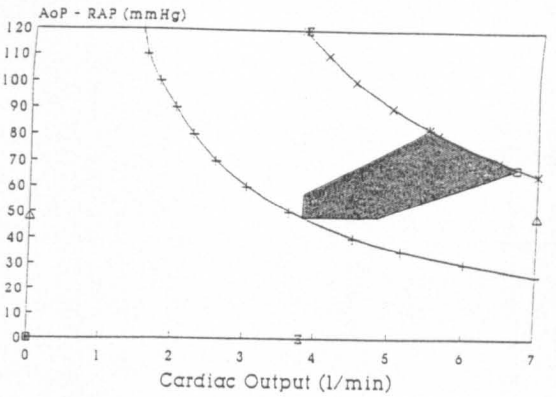


FIGURE 5 Combined criteria for left ventricle. *Shaded section* indicates target area. *AoP,* Mean arterial pressure; *RAP,* right atrial pressure.

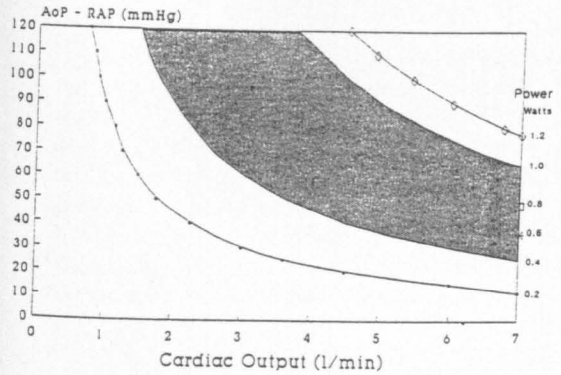


FIGURE 4 Optimum static power output for left ventricle. *Shaded section* indicates target area. *AoP,* Mean arterial pressure; *RAP,* right atrial pressure.

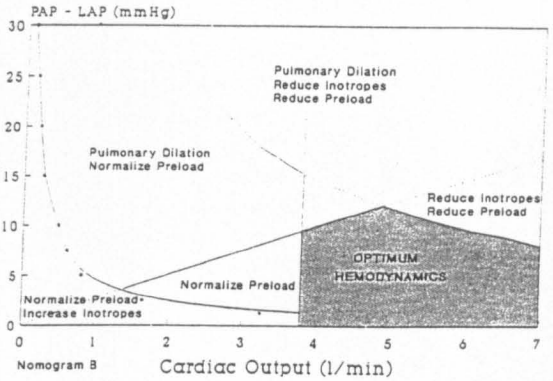


FIGURE 6 Nomogram B: Right ventricular function. *PAP,* Mean pulmonary artery pressure; *LAP,* left atrial pressure.

Figure 5 combines all the previously mentioned criteria for the left ventricle and systemic circulation and indicates the area of optimum hemodynamics for a donor heart.

FORMULATION OF NOMOGRAM B

Nomogram B (Figure 6) describes the hemodynamic state of the right ventricle and pulmonary circulation for a donor of body surface area of 1.8 m^2. The pressure drop across the lungs (Pulmonary Artery Pressure [PAP] – Left Atrial Pressure [LAP]) is plotted on the ordinate against CO on the abscissa.

With left and right ventricles pumping in series, the minimum CO is maintained at 3.8 L/min. The pressure drop across normal lungs is often very small but must be finite for blood to flow from the right ventricle to the left atrium. Because the pressure drop across the lungs should preferentially be as small as possible, the minimum criterion is defined as 0 mm Hg (Figure 7).

Figure 8 indicates lines of constant pulmonary vascular resistance (PVR). This is calculated as follows:

$$\text{PVR (in dynes} \times \text{second per centimeter}^5) = \frac{\text{PAP} - \text{LAP}}{\text{CO}} \times 79.9$$

The maximum normal PVR is selected as 200 dyne × sec/cm^5.

Figure 9 indicates lines of constant right ventricular static power, calculated as follows:

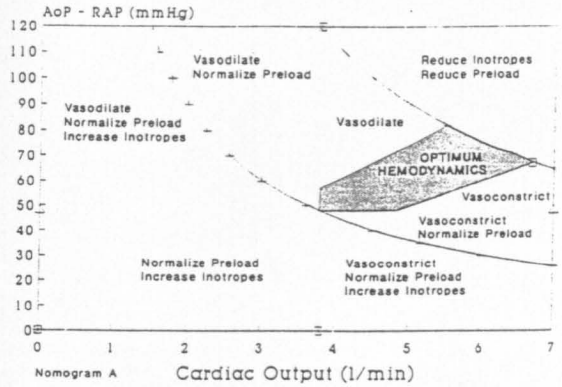


FIGURE 1 Nomogram A: Left ventricular function.

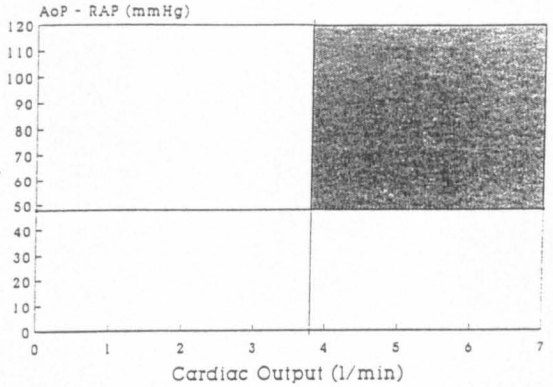


FIGURE 2 Minimum criteria for left ventricle. *Shaded section* indicates target area. *AoP,* Mean arterial pressure; *RAP,* right atrial pressure.

An argument is to be presented with the relationship between developed pressure (Arterial Pressure − Atrial Pressure) and CO, together with optimal resistance and static power for each ventricle. With established minimal criteria for each it is possible to delineate a "target" area for optimum function. By plotting current parameters it is possible accurately to describe function and the disparity between this and the optimum. In addition, it is also simple to predict the intervention required and to use the nomograms to chart clinical responses. As a trend chart the nomogram for the left ventricle (Nomogram A) is used for initial evaluation and provided that a normal range of left atrial pressures are attainable only the left ventricular nomogram is subsequently required. If an element of primary or secondary right-sided heart failure is present, then it is necessary to optimize this with the right ventricular nomogram (Nomogram B) before continuing.

Our thesis is that this approach provides a more focused method for accurately describing current global heart function, measuring and qualifying any disparities, and monitoring the efficacy of any therapeutic intervention.

FORMULATION OF NOMOGRAM A

Nomogram A (Figure 1) describes the hemodynamic state of the left ventricle and systemic circulation for an average-sized donor with a body surface area of 1.8 m^2 (nomograms for individual patients would normally be generated). The pressure drop across the systemic circulation (Arterial Pressure − Right Atrial Pressure [RAP]) is plotted on the ordinate against CO on the abscissa. Each of the following figures has a shaded area that indicates the optimal hemodynamic state for the criterion specified.

Minimum acceptable hemodynamic criteria selected for nomogram A include a pressure drop across the systemic circulation of 48 mm Hg (a minimum mean arterial pressure [MAP] of 60 mm Hg with a maximum RAP of 12 mm Hg) and a CO of 3.8 L/min (equivalent to a cardiac index of 2.1 L/min/m^2) (Figure 2). These minimal criteria are derived from mortality studies of patients with cardiogenic shock and postmyocardial infarct[2,3] and from data from our own donor hemodynamic database.

Figure 3 indicates lines of constant systemic vascular resistance plotted on the same axes. This is calculated as follows:

$$\text{Systemic Vascular Resistance (in dynes} \times \text{second per centimeter}^5) = \frac{\text{MAP} - \text{RAP}}{\text{CO}} \times 79.9$$

The normal hemodynamic range selected is 800 to 1200 dyne × sec/cm^5.

Figure 4 indicates lines of constant left ventricular static power plotted on the same axes. This is calculated as follows:

$$\text{Left Ventricular Static Power (in watts)} = (\text{MAP} - \text{RAP}) \times \text{CO} \times 2.2167 \times 0.001$$

The minimum limit of left ventricular static power is taken as 0.4 W, calculated from the minimum hemodynamic criteria of a MAP of 60 mm Hg, RAP of 12 mm Hg, and CO of 3.8 L/min. A maximum value for left ventricular static power is selected to be 1.0 W; at levels greater than this unnecessary work is being performed by a donor heart.

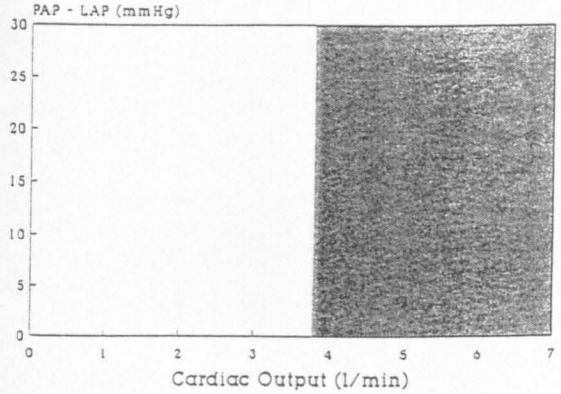


FIGURE 7 Minimum criteria for right ventricle. *Shaded section* indicates target area. *PAP,* Mean pulmonary artery pressure; *LAP,* left atrial pressure.

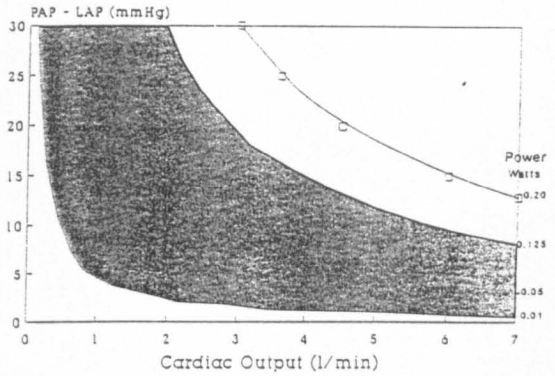


FIGURE 9 Optimum static power output for right ventricle. *Shaded section* indicates target area. *PAP,* Mean pulmonary artery pressure; *LAP,* left atrial pressure.

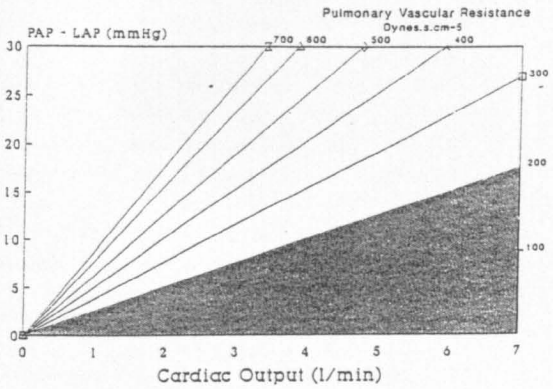


FIGURE 8 Target pulmonary vascular resistance. *Shaded section* indicates target area. *PAP,* Mean pulmonary artery pressure; *LAP,* left atrial pressure.

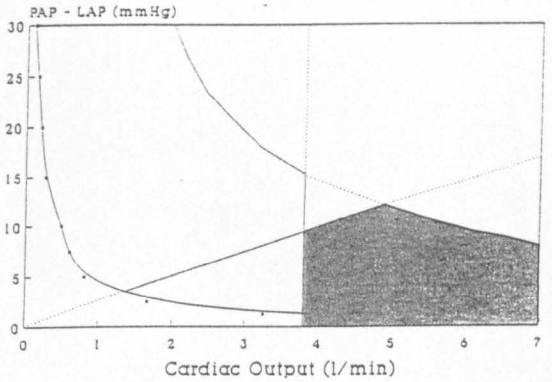


FIGURE 10 Combined criteria for right ventricle. *Shaded section* indicates target area. *PAP,* Mean pulmonary artery pressure; *LAP,* left atrial pressure.

$$\text{Right Ventricular Static Power (in watts)} = (\text{PAP} - \text{LAP}) \times \text{CO} \times 2.2167 \times 0.001$$

Minimum right ventricular static power is estimated as 0.01 W, corresponding to a pressure drop across the lungs of 1 mm Hg with a CO of 3.8 L/min. The maximum right ventricular static power at rest is estimated at 0.125 W, corresponding to a pressure drop of 15 mm Hg across the lungs (maximum acceptable pulmonary pressure drop for orthotopic heart transplantation) with a CO of 3.8 L/min.

Figure 10 combines all the previously mentioned criteria for the right ventricle and pulmonary circulation and indicates the target area of optimum hemodynamics.

DISCUSSION

To display the current status of the donor, it is necessary to plot the pressure drop against the CO for each side of the heart. The resistance and static power output of the ventricle are automatically displayed without any further calculations. Any hemodynamic abnormalities are immediately obvious, with both the magnitude and direction of the donor from the target area being indicated. The trend data show any hemodynamic change since the last set of measurements, and the most appropriate interventional therapy can then be easily determined.

Figure 11 indicates specific interventions and their effects:

1. Adjustment of preload moves the status point across power bands at an angle and magnitude depending on functional integrity.
2. The addition or withdrawal of inotropes has a similar effect but also depends on receptor status.
3. Adjustments to the pulmonary or systemic impedance with the use of dilators or constrictors tend to move the status point along power bands. The magnitude of these changes depends on vascular compliance.

In practice this means that any heart that is producing more than the minimum power output should be capable of moving into the optimum target area by judicious use of dilators or constrictors alone, although optimization of preload is also beneficial. Gross abnormalities of afterload often influences power output by altering the myocardial supply/demand ratio, so that optimization may cause shifts across power bands in the first instance.

If minimum power outputs are unattainable at maximum preloads of 12 to 15 mm Hg, then inotropes are indicated. Our criteria exclude any heart for transplantation that cannot produce the minimum power level with a preload of 15 mm Hg with less than 5 μg/kg/min of dopamine after optimum management. Visual inspection of the donor heart still plays an important role in excluding wall motion abnormalities and hearts with coronary artery disease. Obviously, some hearts can be excluded if the donor's hospital is able to perform an ultrasound study before the donor team arrives.

For full assessment the heart can be volume loaded to determine the response to preload changes. Hearts can then be compared by looking at the static power at a preload of 10 mm Hg. The response to volume loading can then be examined by the gradient of the static power curve at a preload of 10 mm Hg. This technique allows a heart to be compared in both the donor and recipient and for the functional comparison of different hearts.

Myocardial overdrive, in situations where left ventricular outputs exceed about 1 W at rest, usually represents inappropriate management for a heart that is about to receive ischemic trauma. In these cases inotropes can be reduced and the preload lowered.

Nomograms A and B contain suggested treatment strategies, in order of priority, for donors in each hemodynamic territory. It is normally necessary to use only Nomogram A to follow the correction of any gross discrepancies. However, if right-sided heart dysfunction is significant (primary or secondary to an elevated PVR), then Nomogram B is first used to optimize right-sided function.

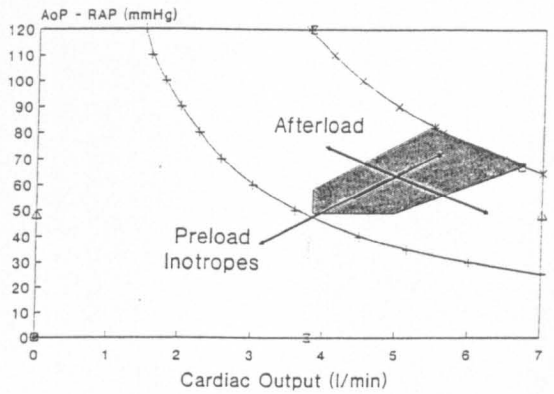


FIGURE 11 Effects of specific interventions. *AoP*, Mean arterial pressure; *RAP*, right atrial pressure.

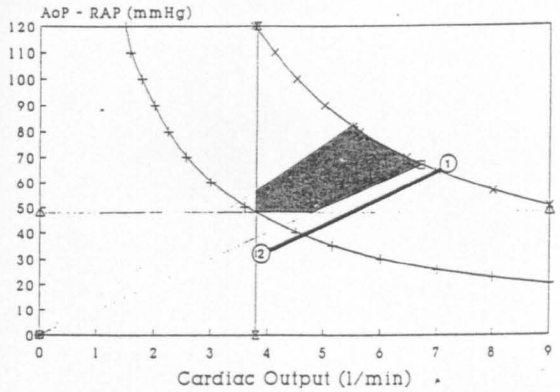
At our institution all donors are monitored with an invasive arterial pressure line and a balloon-tipped pulmonary artery catheter. Using the nomogram approach, together with our donor management techniques,[4] we have retrieved and successfully transplanted many organs that would otherwise have been refused on referral.[5]

The nomograms used are specific to patient size, because they are constructed according to actual CO data. This avoids the use of standardized index data, which are less commonly used within critical care environs. The only difference between sized nomograms is the value for the minimum CO, which is adjusted to a cardiac index of 2.1 L/min/m^2 for each patient.

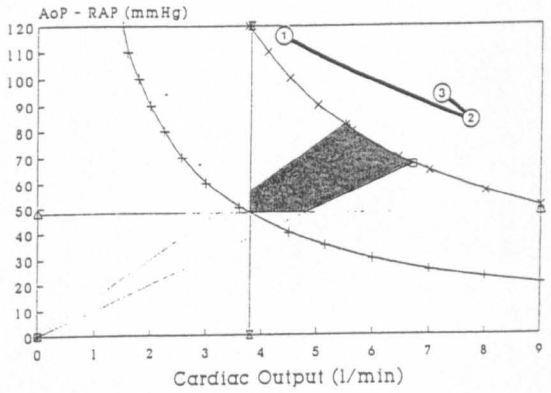
This nomogram technique has a general utility within the critical care environment. A similar nomogram approach for oxygen delivery is being developed.

CONCLUSION

We contend that the nomogram approach to myocardial functional characterization and therapeutic guidance provides a simple and effective method of evaluating the function of a heart. This is particularly important with the retrieval of donor hearts, where confidence of good postimplantation function is essential. Hearts can be extensively evaluated with preload, afterload, and inotropic changes so that only hearts with severe functional impairment are refused for transplantation. The technique can also be applied to the treatment of any critically ill patient where differential diagnosis becomes less



EXAMPLE 1 Inotropic change. *AoP,* Mean arterial pressure; *RAP,* right atrial pressure.



EXAMPLE 2 Vasodilation. *AoP,* Mean arterial pressure; *RAP,* right atrial pressure.

complicated and ambiguous, the effects of therapeutic intervention are easier for medical and nursing staff to comprehend, and trend plots provide a useful guide to patient progress.

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APPENDIX

The following examples involve multiorgan donors. All were at distant hospitals and were therefore not being treated by our team until arrival, when a pulmonary artery catheter was inserted. The examples chosen indicate the changes in hemodynamic status after only one alteration in treatment between readings.

Example 1: Inotropic Change

A 25-year-old man had been admitted and ventilated for 30 hours after a subarachnoid hemorrhage. He had a chest infection and ECG changes, including T-wave inversion in lead I and aVL, and was receiving 8 μg/kg/min of dopamine and 17 μg/kg/min of dobutamine at time of referral.

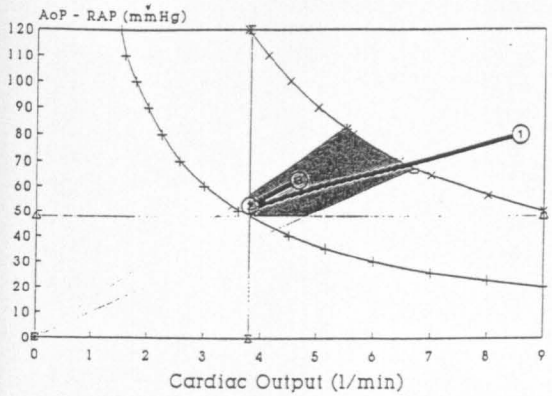
When a pulmonary artery catheter was placed the patient had pulmonary capillary wedge pressure of 17 mm Hg and required 6 μg/kg/min of dopamine and 6 μg/kg/min of dobutamine *(point 1).* The patient showed inotrope dependency with hemodynamic profile *(point 2)* on temporary withdrawal of all inotropes with the same preload. The heart was therefore deemed unsuitable for transplantation and was retrieved for valves only. Note that function moved *across* power lines.

Example 2: Vasodilation

A 48-year-old woman had a massive extradural hemorrhage and right intracranial hemorrhage after she fell at home. She had been in the hospital for 12 hours before pulmonary artery catheterization.

On inspection the heart was working extremely well with pulmonary capillary wedge pressure of 4 mm Hg and no inotropic support, although systemic vascular resistance was 2000 dyne × sec/cm^5 *(point 1).* Sodium nitroprusside was initiated at 1.4 μg/kg/min and prostacyclin at 4.8 ng/kg/min. This reduced systemic vascular resistance to 870 dyne × sec/cm^5 *(point 2),* with slight improvement in power. Sodium nitroprusside dosage was reduced to 0.9 μg/kg/min, with prostacyclin dosage held constant. This therapy had the effect of changing systemic vascular resistance back to 1000 dyne × sec/cm^5 *(point 3),* increasing power again by a small amount.

This is an example of a heart with normal function. Vasodilation causes movement *down* power line with some minimal increase in power because of improved supply/demand ratio.



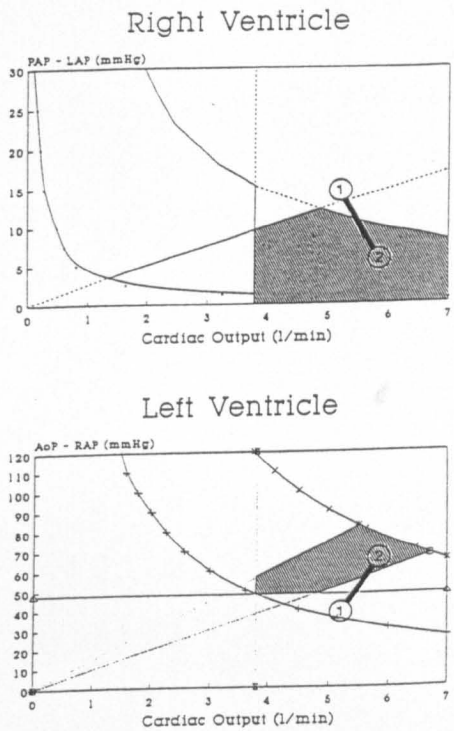
EXAMPLE 3 Preload change. *AoP,* Mean arterial pressure; *RAP,* right atrial pressure.

Example 3: Preload Change

A 17-year-old male patient had been at the hospital for 80 hours with fractured skull after a traffic accident. On full monitoring pulmonary capillary wedge pressure was 23 mm Hg with the heart at the top of the function curve *(point 1).* Overenthusiastic venisection reduced pulmonary capillary wedge pressure to 9 mm Hg *(point 2),* causing the hemodynamic profile to be just acceptable. Additional volume increased the pulmonary capillary wedge pressure to 11 mm Hg, moving the hemodynamic profile into the middle of optimum band *(point 3).* These volume changes moved the hemodynamic profile across power bands and provided a simple method of hemodynamic optimization in patients with normal cardiac function.

Example 4: Elevated Pulmonary Vascular Resistance (Hypoxic)

A 19-year-old woman had been involved in a traffic accident and had required resuscitation by an ambulance crew. Extensive hemorrhage had occurred because of a lacerated liver, and 12 U of blood and fresh-frozen plasma were transfused. On inspection right atrial pressure was 25 mm Hg with pulmonary capillary wedge of 11 mm Hg and PVR



EXAMPLE 4 Elevated pulmonary vascular resistance (hypoxic). *PAP,* Mean pulmonary artery pressure; *LAP,* left arterial pressure; *AoP,* mean arterial pressure; *RAP,* right arterial pressure.

of 240 dyne × sec/cm^5. Arterial saturation was 79% with fractional oxygen concentration in inspired gas of 100%. Obvious right ventricular failure resulted from elevated PVR, probably caused by hypoxia *(point 1).* The airway was cleared, resulting in an increase in arterial saturation from 79% to 99.8%, leading to acute drop in PVR to 80 dyne × sec/cm^5. Right ventricular function improved dramatically, with an RAP of 13 mm Hg and pulmonary capillary wedge pressure of 10 mm Hg, and left ventricular hemodynamic profile moved steeply up across power lines *(point 2)* into the target area.